ASBMT Poster Presentations

1

RAPID SEQUENCE TANDEM TRANSPLANT FOR CHILDREN WITH METASTATIC SARCOMA

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Patients with metastatic sarcoma have a poor prognosis, with disease-free survival (DFS) of 10-25%. We have piloted an intensive protocol with 5 cycles of induction chemotherapy, followed by tandem stem cell transplant, 4-6 weeks apart. Stem cell collection was performed after the third or fourth cycle of induction chemotherapy. Local control was managed with either surgery or local radiation therapy.

Results: 28 patients were enrolled: 16 patients-Ewing's sarcoma (ES); 8 patients- rhabdomyosarcoma (RMS); 2 patients-undifferentiated sarcoma (UDS); 2 patients-synovial sarcoma (SS). Except for 3 ES early in therapy, all other patients are > 1 year beyond transplant. Mean follow-up is 32.5 months after completion of the second transplant. 25/28 patients demonstrated adequate initial response, qualifying to proceed to transplant. Three patients showed evidence of progressive disease through the induction cycles and did not proceed. All patients undergoing transplant tolerated the first transplant without undue toxicity. One patient with RMS developed progressive disease after HD#1, and did not proceed to HD#2. 24/28 patients proceeded to the second transplant within 42 days. Engraftment occurred rapidly in both transplants with a median time to neutrophil engraftment of 11 days after HD #1 and 12 days after HD#2. DFS was 30% at 3 years for all patients (90%CI, 14-46%) (n=28). Patients with ES had a significantly better outcome compared with non-ES patients (56%vs 8% p<.013).

Conclusion: Using an intensive tandem transplant approach to patients with metastatic sarcomas is feasible and well tolerated, even in very young children. Patients with ES have a significantly better outcome than patients with non-ES.

2

TREATMENT WITH HIGH DOSE PULSE STEROID IN CHRONIC GRAFT-VERSUS-HOST DISEASE

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Corticosteroids remain essential for the initial treatment of active chronic graft-versus-host disease (cGVHD). However, the optimum dose and schedule is unknown. We have reviewed our results on 41 patients treated with a high-dose pulse steroid regimen (PST) at a dose of 10 mg/kg/day for 4 consecutive days. After 4 days, all patients received a course of systemic immunosuppressive therapy. Thirty-four patients (85%) had failed a median of 2 (1-5) treatment regimens prior to the PST. Twenty-two of 41 (54%) patients evaluable for response showed a major improvement in all cGVHD manifestations including softening of the skin, increased range of motion, and improved performance status while 9 (22%) showed a minor improvement, defined as improvement in cGVHD in some sites but not in all. According to univariate analysis, only the donor type was associated with response. Twenty-four (86%) patients who had marrow transplant from a sibling donor including one antigen mismatch and 7 of 13 (54%) patients who had matched unrelated donor or allogeneic blood stem cell transplant responded to PST (p=0.049). Three patients developed acute hypertension (n=1) and infection (n=3) following therapy. Eighteen (58%) patients who responded to PST and a subsequent immunosuppressive regimen did not progress after a median follow-up of 25 months (0.3-52). For the PST-responsive group, the median progression free survival (PFS) was 46 months. Univariate analysis revealed that the dose of prednisone that patients receiving prior to PST (<0.5 vs. ≥0.5 mg/kg) [Hazard Ratio (HR)=4.8, p=0.004] and the type of onset of cGVHD (progressive vs. denovo/quiescent) (HR=3.5, p=0.08) were associated with PFS. The 2-year probability of PFS was 77% (50-91%) and 75% (31-93%) for patients who had major and minor improvement after PST, respectively. Our results indicate that PST is an effective and well-tolerated regimen for controlling active cGVHD in patients who failed on previous therapies.

3

HIGH-DOSE SAMARIUM WITH AUTOLOGOUS STEM CELL SUPPORT, A NOVEL TREATMENT OF METASTATIC OR LOCALLY RECURRING OSTEOSARCOMA AND OSTEOBLASTIC BONE METASTASES

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We performed a phase I dose escalation trial using peripheral blood progenitor cell support following high-dose 153Sm-EDTMP in patients with advanced osteosarcoma or osteoblastic bone metastases (N=6); we also report on 5 additional patients after the completion of the study. Total body radioactivity was monitored on day 13 after 153Sm-EDTMP infusion and was always <3600 microCi, a safe level for stem cell infusion. Autologous peripheral blood progenitor cells were given on day 14 after 153Sm-EDTMP. Patients were monitored as outpatients. Transfusion support was required but non-hematologic side effects were not seen until the maximally tolerated dose (MTD)was reached at 30 mCi/kg. At this dose hypocalcemia was seen with the 30 min 153Sm-EDTMP infusion. Lung lesions did not tend to respond to this therapy; responses seemed "bone-specific". Two patients with osteosarcoma had major responses at 6 mCi/kg. One received an estimated dose of 22,000 cGy to an osteoblastic lesion of the left mandible and remains in CR without additional bone metastases >28 months after 153Sm-EDTMP. Another osteogenic sarcoma patient at the 6.0 mCi/kg level had an estimated absorbed radiation dose of 19,300 cGy to a lesion in the left humerus; this patient remains in CR >18 months. At 30 mCi/kg another osteosarcoma patient had dose estimates of 23,800 and 24,100 cGy in two skull metastases. Representative scintographic images will be presented.

Thus, by using autologous peripheral blood progenitor cell support, high-dose 153Sm-EDTMP (with or without external beam radiation) may provide a new means to more effectively provide very high and potentially curative doses of radiation to solid tumors involving bone, especially osteosarcoma. A phase II study of 153Sm-EDTMP at 30 mCi/kg in locally recurring and metastatic osteosarcoma is planned.

4

THE EFFECTS OF TOTAL OUTPATIENT AUTOLOGOUS STEM CELL TRANSPLANTATION FOR BREAST CANCER ON RESOURCE UTILIZATION

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Introduction: 48 patients who received autologous stem cell transplantation for breast cancer at Scripps Clinic between June 1997 and February 1999 participated in a longitudinal study of total outpatient transplantation. This abstract focuses on outcome measures and reasons and rates of readmission.

Patients: 45/48 had sufficient data for analysis. Inpatient status was reserved for patients without an available caregiver. Local accomodations were arranged for patients who desired outpatient therapy yet lived greater than 30 minutes from the BMT unit.

Terminology: Graduation Date = date at which care was returned to the referring oncologist; Total Program Days = number of days between the first date of high-dose chemotherapy and the graduation date; Outpatient days also included days between inpatient discharge date and graduation date.

Results: For all 45 patients, the average number of total program days was 36. The average total number of days of antibiotic use was 12. There was no regimen or disease related mortality while they were on the program. 35/45 patients on study (77%) were treated as outpatients. 11/35 outpatients (31%) had to be readmitted; 1 for social reasons, 1 for neutropenic fever, 2 for bacteremia, and 7 for regimen-related toxicity. Only 1/21 outpatients who developed neutropenic fever was admitted. Even with readmis-

sions, the outpatient group had significantly fewer (p = 0.02) average total program days (34) than the initial inpatient group (42). The readmitted outpatient group had significantly fewer (p = 0.03) average inpatient days (8) than the initial inpatient group (14). Antibiotic usage patterns were not significantly different between groups.

Discussion: Patients who began their transplantation therapy as outpatients had less average total program days and inpatient days than their inpatient counterparts. Neutropenic fever in this population could be effectively managed on an outpatient basis. Intergroup comparisons of economics and quality of life are underway.

5

TRASTUZUMAB FOLLOWING HIGH DOSE CHEMOTHERAPY WITH STEM CELL RESCUE FOR PATIENTS WITH METASTATIC BREAST CANCER

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The use of high dose chemotherapy with stem cell rescue for treatment of metastatic breast cancer has been effective in generating high rates of response but the large majority of patients subsequently relapse from sites of minimal residual disease. A focus of investigation has been the incorporation of immunotherapeutic strategies in an effort to eliminate persistent chemotherapy resistant disease. In the present study, we are examining the impact of Trastuzumab therapy given weekly for 1 year following stem cell transplantation. 15 patients, who demonstrated response to primary chemotherapy for metastatic disease, have been enrolled between 6/99 and 7/00. At time of transplant, 6 patients had visceral disease involving lung and/or liver, 2 patients had bone only disease and 3 patients achieved complete remission in response to pre-transplant induction chemotherapy. The high dose chemotherapy regimen consisted of cyclophosphamide, thiotepa, or carboplatin (STAMP 5) for 13 patients and cyclophosphamide, BCNU, cisplatin (STAMP 1) for 2 patients. To date, 13 patients have initiated Trastuzumab therapy at a median of 40 days following stem cell infusion. No treatment related mortality has been observed. Trastuzumab was well tolerated with no clinically significant treatment related toxicity noted. Transient anemia, lymphopenia and leukopenia was seen in 2, 3 and 3 patients respectively. No significant cardiac events were noted and the median ejection fraction remained stable following transplant and after 1 and 3 months of Trastuzumab therapy. Of 11 evaluable patients, 7 patients remain free of disease progression with a median of 7 months of follow up and 4 patients have experienced disease progression at a median of 3.5 months following initiation of Trastuzumab. The use of Trastuzumab following high dose hemotherapy has been well tolerated without significant cardiac toxicity. Longer follow up is needed to assess the impact of this treatment strategy on progression free survival.

6

SUCCESSFUL ENGRAFTMENT WITH DISEASE RESPONSE FOLLOWING SIBLING ALLOGRAFT WITH REDUCED CONDITIONING,T-CELL DEPLETION AND DELAYED INCREMENTAL DLI IN CML AND MM

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Stable allogeneic engraftment is achievable following reduced conditioning. We are investigating the engraftment kinetics and Graft versus Host Disease (GVHD) incidence following Fludarabine(Flu) and Melphalan(Mel) conditioning with T depletion of stem cell grafts from matched sibling donors in patients with CML and MM . Twenty three patients (17 MM and 6 CML) median age 53yrs have been enrolled in two cohorts.Cohort I received Flu 150mg/m^2, Mel 140mg/m^2 and at least 4x10^6 CD34+/kg Isolex 300i separated filgrastim mobilised blood cells. Cyclosporin 3mg/kg/day IV started on day -5. Four incremental doses of DLI (3x10^6/kg to 1x10^8/kg CD3+ cells) were scheduled at 2,4,6 and 8 mths. Cohort II received a lower Mel dose (100mg/m^2) and higher day zero T-cell dose (1x10^6 /kg CD3+ cells). Eleven patients were enrolled to Cohort I, twelve to cohort II. Mobilisation failed in 3 donors. 20/23 patients were transplanted with a median 4.2x10^6 CD34 cells/kg. 17/20 patients are analysable. Prompt hematopoietic recovery occurred in 16/17. 9/17 are analysable beyond 6mths. 5/17 died prior to 6mths and 3/17 are alive with <6mths follow up. Two of 5 early deaths followed graft failure in patients with MM hence the protocol modifications. Analysis of sorted myeloid (CD13+) and lymphoid (CD3+) cells has shown high levels of donor myeloid and lymphoid chimerism beyond 6mths in 6/9,low levels in 2/9 and 1/9 has mixed chimerism. Acute GVHD has occurred in 6/17, three prior to (patients in cohort II one dying of Grade 4 GVHD) and three following DLI (all steroid responsive). High level donor T-cell chimerism has been associated with disease response however three patients progressed (2 MM,1 CML) following initial responses. This study using a uniform protocol of T-cell depletion and delayed T-cell add-back confirms that stable engraftment with disease response is achievable using this strategy.

7

ATG DURING CONDITIONING FOR MUD BMT IN ADULTS: PHARMACOKINETICS AND IMMUNE RECONSTITUTION

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We prospectively studied the pharmacokinetics of ATG and immune reconstitution in 37 adult recipients of MUD BMT. 18 males, 19 females, median age 39 years, were grafted with unmanipulated bone marrow after standard conditioning complemented by 4 doses of Thymoglobuline(R) 2.5 mg/kg BW days -5 to -2. Residual serum rabbit IgG (RIG) (ELISA), specific anti-lymphocyte-antibodies (ATGeq) (FACS) and lymphocyte subpopulations (FACS) were monitored serially with a median observation time of 526 days. Serum RIG peaks day 0 at a median of 65 µg/mL, and is measured at 10 µg/mL week 8. ATGeq rises to a median of 7.4 µg/mL and is detected at 0.3 µg/mL week 4 after BMT. Rapid and profound lymphocyte depletion is seen during ATG infusion. Whereas NK-cell restoration is achieved within 4 weeks, T- and B-cells are deeply and durably depressed. A first wave of CD3- and CD8-cells emerges around day 100 (median of 114 and 65 /µL), a second around month 9 when CD3- and CD8-cells reach twice those after fully in vitro T-cell-depleted (TCD) BMT (median of 952 and 518/µL) (1). CD4-cell reconstitution is particularly impaired (35/µL day 100) and parallels that after TCD until month 9 when counts increase to a median of 194/µL. CD4/CD45RA/CD45R0-ratio at that timepoint is still as low as after TCD (0.08) and only increases to a relative maximum of 1.3 more than 1.5 years after BMT. At day 100 a median of 77% of CD3-positive and 23% of CD3-negative cells are coated by RIG. Conclusions: Residual ATG circulates for weeks after grafting. Lymphocytes covered with ATG can be detected even longer. During the first year T-cells reconstitute almost completely by peripheral expansion, including CD4-cells. In adults a limited thymic rebound is only seen during the second year after MUD BMT and ATG.

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8

INVOLVED FIELD RADIOTHERAPY (IF-RT) AND BVAC WITH AUTOLOGOUS STEM CELL TRANSPLANTATION FOR REFRACTORY OR RELAPSED NHL

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Several HDC regimens with autologous SCT have been shown to be effective for NHL in chemosensitive relapse and CR2.

Between 8/87 and 4/99, 71 patients with primary refractory or relapsed NHL received BCNU 600 mg/m^2, VP16 400 mg/m^2 x 4, Ara-C 3 g/m^2 x 8, cyclophosphamide 90 mg/kg and autologous SCT. Patients with adenopathy >2.5 cm received IF-RT (2000 cGy) to bulk disease. Our patients were a particularly poor risk group. Median age was 52 (21%>60 year old), 55% had induction failure, 7% CR at time of SCT, 56% refractory disease, 73% ≥2 regimens before SCT and 84% intermediate or high grade NHL. Thirty-two (45%) patients received IF-RT. There was no difference in high-risk patients and those receiving >1 prior regimen between the groups that received IF-RT and no IF-RT. Median follow-up is 699 days. Transplant mortality (d100) was 25% (mostly VOD or pulmonary) and not influenced by age±60. Five year OS and EFS were 44%±13% and 35%±13%. Age ≥60 adversely influenced OS (p=.023) and EFS (p=.006). Histology, disease status at transplant (ABMTR defined), number of regimens and CR1 duration didn't affect OS or EFS. Patients in IF-RT group had improved OS 49%±21% vs. 33%±17% (p<.015) and marginally better EFS 39%±20% vs. 30%±15% (p=.06). Overall relapse rate in 53 evaluable patients was 40%. Time to latest relapse was 2048 days (5.6 years). BVAC/IF-RT was highly effective even in patients deemed poor candidates for autologous SCT. Toxicities of BVAC IF-RT limit its application for patients with liver and lung co-morbidities and may not justify its use in patients with good prognostic indicators i.e. first chemosensitive relapse, low LDH and low tumor burden. Further studies using BVAC IF-RT for patients with poor prognostic indicators seem warranted.

9

HEMATOPOIETIC CELL TRANSPLANTATION FOR AUTOIMMUNE DISEASES: STUDIES IN NOD MICE

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It has been shown that transplantation of MHC-mismatched allogeneic whole bone marrow cells can block the pathogenesis of autoimmune diseases. However, little data exist that demonstrate the effects of clinically relevant hematopoietic cell grafts on autoimmune disease outcome. These grafts include autologous/syngeneic, haploidentical and MHC-matched (mHC mismatched) sources. The goal of our studies is to find the optimal graft composition for hematopoietic cell transplantation (HCT), which blocks the autoimmune process and prohibits recurrence of disease after transplantation with limited morbidity and mortality.

In prior studies we showed that transplantation of MHC-mismatched purified hematopoietic stem cells from AKR/J mice prevents the onset of autoimmune diabetes in prediabetic non-obese diabetic (NOD) mice. Here, we show that HCT of (pseudo)autologous NOD.Thy1.1 and haploidentical donor cells (NODxAKR)F1 do not block the onset of diabetes in prediabetic NOD mice, whereas transplantation of MHC-matched unrelated donor hematopoietic cells, C57BL/6.H-2g7 confer protection. These findings are surprising given that the (NODxAKR)F1 cells express (at a one gene dose) a presumably protective MHC allele, whereas cells from the C57BL/6.H-2g7 donors express the disease associated MHC allele. The data suggest non-MHC background genes expressed in donor derived hematopoietic cells can block autoimmune disease pathogenesis. The human correlates of these studies are matched unrelated donor transplants, which are routinely performed in patients with malignancies and bone marrow failure states.

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PROTECTION AGAINST OPPORTUNISTIC PATHOGENS IN A MURINE MODEL OF HEMATOPOIETIC CELL TRANSPLANTATION USING NEWLY CHARACTERIZED MYELOID PROGENITORS

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Infectious complications in the early posttransplant period remain a significant challenge. Two pathogens associated with neutropenia and share high mortality rates are the fungus *Aspergillus fumigatus* and the virulent gram-negative bacteria, *Pseudomonas aeruginosa*. Weissman, et al, recently identified lineage-restricted progenitors. Two of these, the common myeloid progenitor (CMP) and the further restricted granulocyte-monocyte progenitor (GMP) differentiate into their mature progeny as early as day 6 posttransplant (D+6). We postulated that accelerating reconstitution of functional neutropenia by the inclusion of newly identified myelomonocytic progenitors would significantly reduce morbidity and mortality from these as related pathogens. We established a murine model of infection following HSC transplantation. Groups of mice received syngeneic grafts containing : (1) 200 HSC only or (2) 200 HSC + 1x10^4 CMP and 2x10^4 GMP.

Protection against invasive aspergillosis. Following lethal challenge with 100 conidia of *Aspergillus fumigatus* i.v. on D+3, none of the animals survived. However, 75% of the HSC+CMP/GMP animals survived compared to 0% in the HSC group if challenged on D+7. Fungal colonies were routinely cultured from homogenized organs of moribund mice, whereas cultures from healthy animals yielded no colonies. In an attempt to further shorten the period of susceptibility to *A. fumigatus*, hG-CSF (250 µg/kg) was administered to both groups for five days beginning on D+1. Following a D+3 infection, the survival rate of animals cotransplanted with CMP/GMP was 50% while none of the HSC only group survived.

Protection against *Pseudomonas aeruginosa*. Mice were injected with 300 CFU of *Pseudomonas aeruginosa* i.p on D+7. 75% of the HSC+CMP/GMP group survived compared to 10% survival in the HSC only group. Bacterial load as determined by bioluminescence imaging and culture of homogenized organs correlated with clinically apparent illness and survival.

Therefore, cotransplantation of myeloid progenitors protects against lethal challenge with virulent opportunistic pathogens.

11

INCREASED RISK OF GRAFT-FAILURE AFTER NONMYELOABLATIVE CONDITIONING AND ALLOGENEIC STEM CELL TRANSPLANTATION FROM UNRELATED DONORS

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A fludarabine-based nonmyeloablative preparative regimen was investigated in 42 patients with hematologic malignancies receiving blood stem cell grafts from unrelated volunteer donors. Recipient conditioning consisted of fludarabine 30 mg/sqm on days -6 to -2 and intravenous busulfan 3.3 mg/kg on days -6- to -5. Antithymocyte globuline was added at 2.5 mg/kg i.v. on days -5 to -2. The patients were grafted with bone marrow (n=13) or peripheral blood stem cells either unmanipulated (n=20) or CD34+ selected (n=9). Graft-versus-Host disease prophylaxis was performed with cyclosporin A (CsA, n=12), CsA/methotrexate (n=12) or CsA/mycophenolate mofetil (n=18).

With a median follow-up of 13 months (range, 5-26 months) the actuarial disease-free survival is 64% and 41% for patients with lymphoid malignancies and standard-risk leukemia compared to only 14% for patients with high-risk disease. The main cause of treatment-failure was relapse of disease in high-risk patients (14/42). The rate of primary (n=1) or secondary graft-failure (n=8) was higher after unrelated transplants (21%) compared to the experience with related donors. Univariate analysis indicates a higher rate of graft-failure in patients with chronic myeloid leukemia (p=0.08) and in transplants with one or more HLA mismatches (p=0.09). Chimerism analysis of peripheral blood cell subsets showed an impaired increase of donor NK cell chimerism in patients without sustained engraftment.

Unrelated transplants after dosed reduced conditioning are associated with a higher risk of graft-failure. Pretransplant host immunosuppression has to be optimized to overcome resistance to grafts from unrelated donors after nonmyeloablative conditioning therapy.

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HIGH DOSE THERAPY WITH CD34 SELECTED STEM CELL RESCUE FOR MULTIPLE MYELOMA RESULTS IN PROLONGED PLATELET RECOVERY

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We have treated 20 patients with multiple myeloma (19) or amyloidosis (1) with high dose melphalan +/-XRT and CD34 selected stem cell rescue. Patients with disease responsive to conventional dose induction therapy underwent chemomobilization with Cytoxan 2 gm/m^2/d x 2 alone(16 pts) or with etoposide 100mg/m^2/d x 3 (4 pts); followed byG-CSF/GM-CSF (9 pts) or G-CSF (11 pts). Patients underwent apheresis when the WBC exceeded 1000/uL with a target collection of 6.5 x 10^6 CD34+ cells/kg (1.5 x 10^6 for backup and 5.0 x 10^6 for selection). CD34+ cells were isolated using the Isolex® 300*i* device (Version 1.12, Nexell, Inc). Median age was 57 yrs (41-67 yrs), male/female 10/10. Median number of phereses: 2 (1-7), median CD34 cells collected 18.3 x 10^6, median CD34 frozen for backup:1.5 x 10^6/kg; median CD34+ cells/kg infused post selection: 7.65 x 10^6 (range 0.7-37.2). Median % recovery of CD34+ cells was 51% (14-77%). Patients received melphalan 200 mg/m^2 plus TBI (7pts). One pt was not treated due to rapidly progressive disease. 19 pts completed high dose therapy with CD34 selected stem cell reinfusion. There was one treatment related death (aspiration pneumonia/sepsis). Median follow-up is 12 mos (2-20 mos), 16 pts are alive, 4 expired: pneumonia (1), progressive disease (2), TRM (1). Days to AGC 500/uL: 17 (6-50 days), days to platelet transfusion independence: 55 (22-230 d) for the 14 pts who have recovered plts (too early-2, expired-3, not treated-1). These pts required an average of 20 plt transfusions in the 100 days post transplant. 3 have relapsed, 15/20 are alive relapse-free. This treatment approach is well tolerated but results in prolonged plt recovery despite adequate numbers of CD34+ cells infused.

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HIGH DOSE THERAPY WITH CD34 SELECTED STEM CELL RESCUE FOR NON-HODGKIN'S LYMPHOMA, SLOW RECOVERY OF GRANULOCYTES AND PLATELETS

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We have treated 20 patients with non-Hodgkin's lymphoma (NHL) with high dose chemotherapy +/-XRT and CD34 selected stem cell rescue. Patients with disease responsive to conventional dose induction therapy underwent chemomobilization with Cytoxan 2 gm/m^2/d x 2 alone(13 pts) or with etoposide 100mg/m^2/d x 3 (3 pts); followed byG-CSF/GM-CSF (9 pts) or G-CSF (11 pts). Patients underwent apheresis when the WBC exceeded 1000/uL with a target collection of 6.5 x 10^6 CD34+ cells/kg (1.5 x 10^6 for backup and 5.0 x 10^6 for selection). CD34+ cells were isolated using the Isolex® 300*i* device (Version 1.12, Nexell, Inc). Median age was 48 yrs (21-68 yrs), male/female 12/8. Median number of phereses: 4 (1-9), median CD34 cells collected: 8.6 x 10^6/kg (5.7-67.4); median CD34 frozen for backup: 1.5 x 10^6/kg; median CD34+ cells/kg infused post selection 3.3 x 10^6/kg (2.0-20.6). Median % recovery of CD34+ cells was 51% (30-87). One patient required a bone marrow harvest as well as stem cell collection and nine required 2 selection procedures. Patients received BEAM (11 pts) or Cytoxan/VP-16 plus TBI (8 pts). 19/20 pts completed high dose therapy with CD34 selected stem cell reinfusion. There were no treatment related deaths. Median follow-up is 12 mos (1-19 mos), 14/20 pts are alive, 6 expired: all from PD. Days to AGC 500/uL: 20d (14-102d), days to platelet transfusion independence: 33 (18-101) for the pts who recovered plts (3 exp-ired without plt receovery). 9 have relapsed, 11 are alive relapse-free. This treatment approach is well tolerated with no treatment related mortality but recovery of granulocytes and platelets appears delayed in comparison to unmanipulated stem cell transplants.

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TRANSIENT HYPERBILIRUBINEMIA FOLLOWING PACKED RED BLOOD CELL TRANSFUSIONS (PRBCT) IN PATIENTS UNDERGOING HIGH DOSE CHEMOTHERAPY (HDC) ± AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANT (HSCT)

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Background: Total bilirubin (TB) is measured in HDC ± HSCT patients to detect complications such as VOD, gall bladder disease, drug effects and disease progression. Unexpected hyperbilirubinemia in patients receiving PRBCT following HDC confounds the assessment of potentially serious complications. Methods: A retrospective chart review was performed for patients receiving HDC ± autologous HSCT during 1999. Sixty-one (61) patients were evaluated, 35 (57%) had received HDC with HSCT, 26 (43%) had received HDC only. Median age was 43 (range 21-63) with 36 females and 25 males. Results: Increases in TB to above the upper limit of normal the day following PRBCT were observed in 27 (44%) of the patients evaluated. Doubling of the TB occurred in 14 (23%) of the patients with 6 experiencing greater than one episode of hyperbilirubinemia. TB normalized prior to the next PRBCT in 25 (93%) of patients. The median difference in TB (pre to post PRBCT) for those experiencing hyperbilirubinemia was 0.7 gm/dl (range 0.5-2.4). Median days from last chemotherapy to first hyperbilirubinemia following PRBCT was 9 (range 3-30). Co-existing medical events possibly related to hyperbilirubinemia were seen in 11 (41%) of patients. Continuous infusion narcotics were in use at the time of hyperbilirubinemia in 11 (41%) of the patients.

Conclusion: The normalization of TB following post transfusion hyperbilirubinemia in 93% of these patients coupled with the lack of other proven causes for the hyperbilirubinemia suggests that PRBCT may cause transient hyperbilirubinemia. No significant differences were noted between the two populations in regard to age, gender, days from HDC to first PRBCT, hemoglobin level at PRBCT, HSCT source or disease. Transient hyperbilirubinemia following PRBCT appears to be common in this setting. For clinically stable patients, 24 - 48 hours should be allowed for normalization of TB prior to extensive investigation of other causes.

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ACYCLOVIR VERSUS GANCICLOVIR FOR PREVENTION OF CYTOMEGALOVIRUS ANTIGENEMIA AND DISEASE AFTER ALLOGENEIC TRANSPLANTATION: SIMILAR EFFICACY AND TOXICITY

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The aim of this prospective, randomized trial was to compare the effectiveness of acyclovir (ACV) versus ganciclovir (GCV) in preventing CMV antigenemia and disease, and to determine toxicity. From 1995-1999, 91 CMV seropositive recipients of related and unrelated donor transplants were enrolled. All received GCV 5 mg/kg IV every 12 hrs days -7 to -2, then ACV 10 mg/kg IV every 8 hrs day -1 until ANC ≥ 750/ul. Patients were then randomly assigned to ACV 800 mg (adults)/18 mg/kg (children) orally 5 times a day (n=46) or GCV 5 mg/kg IV weekdays (n=45) until day 100. Any degree of antigenemia was treated with GCV 5 mg/kg IV twice a day for 2 weeks, then 5 mg/kg IV weekdays for 6 weeks. At twelve months, the cumulative incidence of antigenemia was 43% (95% C.I. 28-58%) and 33% (95% C.I. 19-49%) for ACV and GCV prophylaxis, respectively (P=0.24). Adjusted multivariate regression confirmed similar prophylactic efficacy. In the ACV group, 8/46 (11%, 95% C.I. 2-20%) patients developed CMV disease, compared to 5/45 (7%, 95% C.I .0-14%) in the GCV group (P=0.39); time to development of disease was similar. Multiple regression analysis identified ANC ≤ 1500/ul at randomization (P<0.01) and grade II-IV aGVHD (P=0.06), but not the assigned prophylaxis (P=0.50), as independent risk factors for

CMV disease. Increased neutopenia from baseline was experienced by 19/46 (39%) patients who received ACV, versus 28/45 (62%) who received GCV (P=0.03). The one year estimate of OS was 54% (95% C.I. 40-68%) for ACV and 64% (95% C.I. 50-78%) (P=0.38) for the GCV group. Our data do not support the use of GCV at engraftment for prophylaxis of CMV antigenemia/disease, as high dose oral ACV in the setting of preemptive antiviral therapy results in similar outcomes with lower cost, ease of administration, and without additional toxicity.

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LIPOSOMAL AMPHOTERICIN (AMBISOME®) COMPARED TO AMPHOTERICIN B ± ADDITIONAL FMLP IS ASSOCIATED WITH SIGNIFICANTLY DECREASED PMN AGGREGATION FROM G-CSF/DEXAMETHASONE MOBILIZED ALLOGENEIC (DONOR) PMNS

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Recently, Price et al demonstrated the success of collecting 5-fold more neutrophils by leukopheresis from allogeneic donors (AD) mobilized with G-CSF and dexamethasone (G/D) (Price et al, Blood 95:3302, 2000). The concomitant use of both AD PMN transfusions and amphotericin B in febrile neutropenic transplant recipients is limited in part by the increased incidence of PMN aggregation, pulmonary sequestration, and respiratory distress. New liposomal formulations of amphotericin B (Ambisome®) have been demonstrated to be associated with decreased infusional and systemic toxicity in febrile neutropenic transplant patients compared to amphotericin B (Walsh et al, NEJM 340:764, 1999). We compared the in-vitro effect of amphotericn B versus Ambisome® on PMN aggregation from PMNs isolated from G/D mobilized AD. Briefly, following informed consent and screening, 6 AD were mobilized with G (600 mcg SQ) and D (8 mg PO) 16 hours prior to leukopheresis for neutropenic and septic ABO compatible transplant recipients. PMNs (1 x 10^7/ml) were incubated with amphotericin B (10-100 mcg/ml) and Ambisome® (10-100 mcg/ml) ± FMLP ($2x10^7$ M) and aggregation was evaluated by Δ light transmission (%T) in 5 minutes by standard aggregometry. Ambisome® compared to amphotericin B was associated with significantly less PMN aggregation at 50 mcg/ml (0.5 ± 0.5 vs 26.33 ± 8.33%, n=3, P<0.01) and 100 mcg/ml (0.33 ± 0.33 vs 54.33 ± 5.82 %, n=3, P<0.001). Furthermore, with the PMN agonist, FMLP, Ambisome® was also associated with significantly less aggregation compared to amphotericin B at 100 mcg/ml (18.67 ± 1.45 vs 54.67 ± 2.4%, n=3, P<0.001). In summary, these studies demonstrate that liposomal amphotericin (Ambisome®) compared to amphotericin B is associated with significantly less in vitro PMN aggregation and could possibly be administered concomitantly with mobilized AD PMN transfusions. Future prospective controlled clinical trials will be required to confirm this hypothesis.

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IMPROVED SURVIVAL OF EXTRAOCULAR RETINOBLASTOMA FOLLLOWING COMBINED INTENSIVE THERAPY

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Purpose: Most extraocular retinoblastoma patients manifest mutidrug resistance (MDR) due to p-glycoprotein, and few survive beyond a year when treated with conventional chemotherapy and radiation. We evaluated the use of combined MDR reversal chemotherapy consolidated by high dose therapy with autologous stem cell rescue.

Method: Three patients with retinoblastoma who presented with extensive optic nerve involvement (two with tumor at the resection margin of the optic nerve and one with 9 mm optic nerve involvement beyond the lamina cribrosa) were treated with enucleation, orbital irradiation, 6 cycles of carboplatin, vincristine, and teniposide with cyclosporin (33 mg/ kg) to reverse MDR, intrathecal cytarabine and high dose carboplatin, etoposide and cyclophosphamide with autologous stem cell rescue. Stem cells were obtained from peripheral blood, and were CD34 selected as a de facto tumor depletion.

Results: The therapy was associated with acceptable acute toxicities, and no treatment related deaths. All three patients are surviving event-free at 28, 23 and 14 months following diagnosis.

Conclusions: Children with extensive extraocular retinoblastoma that would have died in the past, may achieve long-term event-free survival with intensive multi-modal therapy.

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COULD DIMETHYL SULFOXIDE (DMSO) BE HARMFUL TO NURSING STAFF DURING PBSC REINFUSION?

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In the last years PBPC reinfusion has become progressively utilized, when PBPC are cryopreserved in DMSO at a concentration 5-10%, which is added before the freezing procedure to prevent ice crystal formation in the cells. DMSO is subsequently eliminated by the patients during and after reinfusion mostly via respiratory mucosa and skin, causing the characteristic smell.

In March 2000 we made a survey within BMT centres of GITMO group to evaluate the following: awareness by the staff about potential side effects, protection procedure and possible toxicity to the nursing staff. A questionnaire was sent to 67 centres of GITMO.

The questions were:

1-whether symptoms were recorded during and after reinfusion

2-whether the nurse was aware of protection procedures

3-whether protections devised were put in place

4-characteristics of aeration of rooms in which the procedure was performed

5-modalities of clearance of waste material after reinfusion

34 questionnaires were appropriately filled out; 7 out of 34 (20.5%) centres apply protocols for the reinfusion and procedures for the protection of the nurse; 12 out of 34 (35.2%)centres apply individual devices for protection; 10 out of 34 (29.5%) have the possibility to aerate the room; 13 out of 34 (38.2)have recorded symptoms during reinfusion (11 headache, 9 nausea, 3 vomiting, 1 dyspnea, 1 ocular symptoms);15 out of 34 (44.1%) centres have a specific procedure for the clearance of waste material. The results indicate the lack of appropriate information for the personnel involved in the procedure.

Literature data do not show evidence of toxicity for the personnel performing or attending the procedure. On the other hand, DMSO is classified as teratogen and mutagenic drug based on mice studies. These data suggest that the utilization of general and individual safety procedures is strongly advised.

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A PILOT STUDY OF THE ORAL ABSORPTION OF ITRACONAZOLE SOLUTION IN BONE MARROW TRANSPLANT (BMT) PATIENTS

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Background: Itraconazole is a reasonable choice for antifungal prophylaxis in allogeneic BMT recipients given its spectrum of activity and its availability as an oral formulation. The absorption of this agent in BMT patients has been questioned. Peak concentrations of itraconazole greater than 250 ng/ml and itraconazole plus hydroxyitraconazole (OHI) greater than 1000 ng/ml via reverse-phase high performance liquid chromatography (HPLC) have been correlated with maximum efficacy. Case reports suggest suboptimal absorption in BMT patients. Objective: To evaluate the bioavailability of itraconazole in a population of allogeneic BMT recipients that would include patients with GVHD. Methods: Patients were instructed to begin itraconazole solution at a dose of 200 mg daily on an empty stomach. Itraconazole and OHI serum concentrations were assayed using reverse-phase HPLC and were obtained 2 hours after the itraconazole dose. Patients with subtherapeutic concentrations had their itraconazole dose increased by 100 mg. Patients were interviewed to determine any adverse experiences with the drug. Results: Eight allogeneic BMT patients were included in the pilot analysis. At least one itraconazole level was obtained on all eight patients. Four of the eight patients were subsequently withdrawn from the study for the fol-

lowing reasons: elevated liver enzymes, engraftment failure, nausea/intolerability and start of amphotericin therapy with only nausea in one patient felt to be an effect of the itraconazole. Three patients had biopsy confirmed GVHD of the gut. Mean concentrations of itraconazole were 2411 ng/ml(range 1252-4807 ng/ml) and OHI were 5305 ng/ml (range 2067-8407 ng/ml). All concentrations achieved the desired target levels and no dosage adjustments were required. The presence of GVHD did not affect serum concentrations of either itraconazole or OHI. Conclusions: Allogeneic BMT patients both with and without GVHD appear to adequately absorb itraconazole after administration of the oral solution. Further study of itraconazole in BMT prophylaxis appears warranted.

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AUTOLOGOUS CD34+ SELECTED HEMATOPOIETIC STEM CELL TRANSPLANTATION (CD34+/HSCT) FOR MULTIPLE SCLEROSIS: UPDATE OF A SINGLE CENTER EXPERIENCE IN 10 PATIENTS

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We analyzed the outcome of the first 10 patients with multiple sclerosis (MS) included in a phase II trial of CD34+/HSCT.

Blood stem cells were mobilized with cyclophosphamide (Cy) (3 g/sqm) and G-CSF (5 ug/kg/d). CD34+ selection was performed by means Isolex 300 or Clinimacs system. Conditioning included BCNU (300 mg/sqm), Cy (150 mg/kg) and ATG (Merieux) (60 mg/kg). Patients were assisted in LAF rooms, received low microbial diet, oral ciprofloxacin, fluconazole and acyclovir, inhaled pentamidine, IgIV, and G-CSF.

Between April 1998 and October 2000, 10 patients with secondary progressive (6 cases) or relapsing/remitting (4 cases) MS underwent HSCT. Their median (range) age, Kurtzke EDSS and Karnofsky score were 30 years (23-45), 6.25 (5-6.5) and 60 (40-80), respectively. The grafts contained a mean (+/-SD) of 4.8 (+/-1.7) $x10^6$ /kg CD34+ cells. Clinimacs selection (8 cases) resulted in a mean of 0.4 (+/-0.19) $x10^4$ /kg CD3+ cells; being 30 and 7 $x10^4$ /kg, respectively in two Isolex procedures. Conditioning-related toxicities were grade I mucositis in all patients and grade II liver toxicity in one. WBC >500/uL and platelet >20.000/uL were achieved after a median of 10.5 (9-18) and 12 (9-15) days, respectively. One patient had two episodes of CMV infection that responded to ganciclovir. After a median follow-up of 18 (6-32) months, three patients have shown an improvement of EDSS (1, 1.5, and 1.5, respectively), two have worsened (0.5 and 1) 30 and 12 months after HSCT, and five have remained stable (including one patient that worsened during HSCT). Two patients had relapses after HSCT. All episodes were subjective sensory symptoms that lasted a few days and did not require treatment. No patients needed additional immunosuppressive therapy after HSCT.

In conclusion, CD34+/HSCT is a feasible approach in MS. Whether CD34+/HSCT influences the clinical course of patients remains to be seen.

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A SINGLE CENTER EXPERIENCE OF UMBILICAL CORD BLOOD TRANSPLANTATION IN CHILDREN IN HONG KONG

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From August 1994 to November 2000, 10 children, aged 2.1 to 12.1 years (median: 6.2 years) with thalassemia (3), leukemias (5)and metabolic conditions (2), received allogeneic umbilical cord blood transplant following myeloablative conditioning therapy in our center. The graft source was derived from five siblings, four HLA-identical, one HLA mismatched at two loci. Unrelated cord blood mismatched for 1 to 2 antigens were also used, 3 from local cord blood bank, 2 from cord blood banks elsewhere. Median recipient body weight was 16.9 kg (range: 11.7 - 38.6). Median volume of the cord blood harvested was 93 ml (range: 44 - 130). There were two subjects receiving neonatal blood and bone marrow in addition respectively as supplement to the cord blood with low cell count. Median values of cells in the remaining umbilical cord blood graft were: nucleated cells $5.07 \bullet 10^7$/kg ($2.90 \bullet 10^7$ - $1.12 \bullet 10^8$); mononuclear cells $2.03 \bullet 10^7$/kg ($4.50 \bullet 10^6$ - $4.72 \bullet 10^7$); CFU-GM $4.59 \bullet 10^4$/kg ($5.96 \bullet 10^3$ - $1.80 \bullet 10^5$); CD34 $1.34 \bullet 10^5$/kg ($2.93 \bullet 10^4$ - $1.03 \bullet 10^6$). For non-malignant conditions, the pre-transplant conditioning regime was busulphan and cyclophosphamide. For malignant conditions, the pre-transplant conditioning regime varied with total body irradiation based(2), and chemotherapy based(3). GVHD prophylaxis included combinations of cyclosporin, methotrexate and methylprednisolone. 2 patients died within 100 days post-transplant due to sepsis. The median time for neutrophil engraftment was 19 days (13 - 32) and 38.5 days (22 - 50) for platelet. The median follow-up period was 20.4 months (range, 0.03 to 6.23)with overall survival of 60%. There were three subjects failed to develop donor engraftment, three had mixed chimerism and four with complete donor chimerism. No GVHD was observed among HLA-identical sibling transplant. One mismatched related and three unrelated transplant subjects had acute GVHD grade II - III. In conclusion, umbilical cord blood transplant is a feasible alternative haematopoietic cell source for transplant.

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CONDITIONING REGIMEN AND GRAFT-VERSUS-HOST DISEASE (GVHD) AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT): INTRAVENOUS BUSULFAN/CYCLOPHOSPHAMIDE (BU/CY) VS TOTAL BODY IRRADIATION (TBI)

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Objective: To compare the impact of IV Bu/Cy vs TBI on acute and chronic GVHD. Methods: We evaluated a total of 84 patients with CML in chronic or accelerated phase who received grafts from HLA-matched related donors. Thirty-two patients had Cy, Thiotepa, TBI and antithymocyte globulin (ATG) as their conditioning (Group 1) and 52 received IV Bu (0.8 mg/kg) and Cy (60 mg/kg) (Group 2). Patients in Group 1 had CD8-depleted grafts (BM=21, PBSC=11) and cyclosporin/methylprednisolone prophylaxis. Patients in Group 2 had unmanipulated transplants (BM=32, PBSC=20), with tracolimus/methotrexate prophylaxis. Results: The overall incidence of acute GVHD Grade II-IV was 47% (n=15) and 17% (n=9) for Groups 1 and 2, respectively (p=0.0036). Skin was the organ most commonly involved in both groups (1=73%, 2=66%), followed by upper and lower GI tract (1=33%, 2=55% and liver (1=13%, 2=33%). Chronic GVHD was observed in 31% of patients in Group 1 and 25% of patients in Group 2 (p=0.53). Mortality for the TBI-treated group is 50% (n=16), compared to 23% (n=12) in Group 2 (p=0.01). The majority of deaths in Group 1 were GVHD-related (68.5%) while they represented only 33% of the deaths in Group 2. The relapse rate rate was 9% (n=3) for Group 1 and 21% (n=11) for Group 2 (p=0.1595). Two patients who relapsed (Group 1) had acute GVHD, and only 3 of 14 relapses in both groups had extensive chronic GVHD. Relapse accounted for 6% of deaths in Group 1 (n=1) and 33% in Group 2 (n=4). Conclusions: In this study, the group of patients receiving TBI had a higher incidence of acute GVHD, higher mortality and higher GVHD-related mortality than patients treated with IV Bu/Cy. The conditioning regimen does not affect the clinical features of acute GVHD or incidence of chronic GVHD.

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A PROSPECTIVE STUDY OF LOW-DOSE ABELCET IN THE TREATMENT OF NEUTROPENIC PATIENTS FAILING OR EXPERIENCING TOXICITY WITH CONVENTIONAL AMPHOTERICIN-B

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The mainstay of anti-fungal therapy is Amphotericin-B. Ampho-B is, however, toxic.Liposomal/lipid-associated prepara-

tions of Ampho-B have been developed to minimise toxicity, but cost is significantly higher. Abelcet is a lipid-complexed preparation of Ampho-B. Recommended dose is 5mg/kg/day, however lower doses are effective. This study investigated the use of low-dose Abelcet (2mg/kg/day) in neutropenic patients failing treatment, or experiencing toxicity with Ampho-B. Antifungal treatment with Ampho-B was initiated in patients who continued to be pyrexial after 72-96 hours of 1st/2nd line antibiotics. Patients failing to respond to Ampho-B, or who developed toxicity received Abelcet 2mg/kg/day. Forty-one patients were recruited (median age 46). Haematological diagnoses were as follows: AML 22 (allograft 7, autograft 2), NHL 5 (autograft 3), myeloma 5 (autograft 4), others 9 (allograft 5, autograft 1). Response endpoints - complete response if pyrexia resolved to <37°C on 3 consecutive days; failure if Abelcet dose was increased, if additional antifungal therapy was required, or if fever persisted despite resolution of neutropenia. CR occurred in 27(66%); fever resolved in a median 14 days (range 1-22). Neutrophil counts remained <0.5x10^9/L for median 14 days, but did resolve in 73% of patients. 11 treatment failures - 4 continuing fever (though in 2 neutrophil counts did not recover), 6 dose of Abelcet increased, and 1 because additional antifungal treatment required. 1 patient died in whom there was no evidence of deep-seated fungal infection. Mean creatinine level at start of Abelcet was 139 (range 47-450 umol/l), and at end of treatment was 136 (range 47-390). 2 patients had increasing creatinine levels on Abelcet; both were also receiving concurrent nephrotoxic drugs. Infusion-related reactions were mainly rigors (n=8) or fever(n=4) and responded to treatment with chlorpheniramine/pethidine. Study demonstrates that Abelcet 2mg/kg/day is effective and well tolerated in neutropenic patients, including transplant patients, failing treatment or experiencing toxicity with AmphoB.

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ROUTINE VS RISK-ADAPTED ADMINISTRATION OF COLONY STIMULATING FACTORS (CSF) FOLLOWING AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANT (AUTOPBSCT)

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Various trials report no benefit of CSF therapy following autoPBSCT; others report modest acceleration in neutrophil recovery and decreases in hospitalization among patients (pts) undergoing autoPBSCT as inpatients. Prior to Oct 99, pts initiated CSF therapy on Day 0 following infusion of autoPBSC (Group 1, n=141). After Oct 99, pts initiated CSF therapy on day 7 following autoPBSC infusion only if the CD34+ cell dose was less than 2.5 x 10^6 /kg (Group 2, n=38). Median age, CD34+ cell doses infused, disease and number of PBSCTs performed in the outpatient setting were similar for both groups. In group 1, 82% of pts received GM-CSF following autoPBSCT, 14% G-CSF, 2% both (not simultaneously), and 2% no CSF therapy. In group 2, 8% of pts received GM-CSF, 3% G-CSF, 3% both (not simultaneously), and 86% no CSF therapy. For pts receiving CSF therapy, the mean number of days of CSF therapy was reduced in group 2 (8 days) compared to group 1 (13 days), $p<0.05$. Pts in group 1 achieved an ANC>500/ul on median d12 vs d14 for pts in group 2, p=0.01. Platelet recovery to 20,000/ul occurred on d18 vs d17 for groups 1 and 2, respectively, p=NS. For groups 1 and 2, the incidence of hospitalization in outpatients was 30% vs 25% (p=NS), respectively, and the duration of hospitalization for inpatients was 21d vs 20d (p=NS), respectively. Documented infections occurred in 23% of pts in group 1 vs 24% in group 2 (p=NS). The median days of IV antibiotics for groups 1 and 2 was 8 and 7 days, respectively, p=NS. Administration of CSFs following autoPBSCT accelerated neutrophil recovery, but did not decrease infection, days of IV antibiotics, or hospitalization. Risk-adapted administration of CSFs following autoPBSCT did not significantly affect clinical outcomes and resulted in an approximate savings of $2000/pt.

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CYTOKINES AND POSTNATAL BOOSTS IMPROVE HEMATOLOGICAL PARAMETERS FOLLOWING IN UTERO TRANSPLANTATION IN β-THALASSEMIC MICE

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We have developed a murine model of allogeneic in utero transplantation in B-thalassemic mice to study chimerism, tolerance, and hematological parameters. We have documented that survival of homozygous fetuses receiving allogeneic fetal liver cells improved by 40% as compared to controls. The percent of reticulocytes in peripheral blood was decreased by a mean of 52.8% (p=0.04), and ektacytometry (osmotic fragility) shift toward normal was observed in 30% of the transplanted animals. Mass spectrometry detected up to 5% of donor α and β globin in transplanted mice, which correlated with hemoglobin chimerism. Hemoglobin levels improved with postnatal SCF/GCSF/EPO injection as compared to control mice (11.58±4.03 transplanted, and 9.92±0.39 controls, p=0.13). Chimerism was tested by FACS analysis over the lifetime of the animals. Donor cells were detected in CD45, CD3, and TER 119 populations. The percent of donor cells varied from 0.01-1.6% and persisted throughout life. In mixed lymphocyte cultures, the response of control mice to donor cells increased 30-40% (as compared to pre-boost response, while the response of transplanted animals remained stable. The percent of donor cells significantly increased following postnatal boosts with donor cells (0.3-6%). Cytotoxicity assays demonstrated higher responses to donor cells in control mice as compared to in utero transplanted animals (at 50:1 effector to target ratios, transplanted animals showed 8.66% target lysis, and controls showed 51.85% target lysis, p=0.0003). This experiment demonstrates that even very low levels of donor chimerism may be sufficient for improvement in hematological parameters. The level of chimerism can increase following the postnatal boosts with donor cells in tolerant animals.This possibility of tolerance induction may allow development of successful non-myeloablative transplant regimens in β-Thalassemic patients.

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MICROCHIMERISM SUSTAINS ACTIVE POSTNATAL IMMUNITY IN MICE PRIMED IN UTERO TO ALLOGENEIC CELLS

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Induction of a high degree of tolerance following prenatal transplantation with allogeneic cells is an ultimate goal which may lead to successful prenatal treatment of congenital disorders. This however was not achieved so far and prenatal transplants are only successful in hosts with defective immune systems, such as severe combined immunodeficiency disorders, but not in hemoglobinopathies and inborn errors of metabolism We have previously demonstrated evidence of postnatal immunity to allogeneic c-kit+ cells from cytokine stimulated peripheral blood stem cells following prenatal transplantation with this cells (manuscript submitted).

In order to further study this new phenomenon, we have performed experiment in which purified murine Sca-1+/lin- cells and c-kit+/Lin- cells of C57Bl/6 mice(H-2b,I-E-) mice were injected into Balb/c (H-2d,I-E+) fetal recipients. It was previously demonstrated that the transplantation of I-E+ donor cells into I-E- recipient strains results in microchimerism only and lack of tolerance.

We found that in both groups only microchimerism (<1% donor cells) was observed, but it persisted up to 23 weeks in mice transplanted with sca-1+ cells and only up to 3 weeks in mice which received c-kit+ cells. This difference in longevity of chimerism was related to different level of in vivo and in vitro responses to donor tissues/cells: mice that had prolonged chimerism, hyperejected donor skin grafts as compared to non-transplanted controls and showed evidence of immune rejection of donor cells in cytotoxicity assay. In contrast, mice with short lived chimerism rejected donor skin grafts at a time similar to controls and showed only minimal immune responses to donor cells in cytotoxicity culture. This results support that active immunity to allogeneic cells may be induced by prenatal transplantation and enhanced by the per-

sistence of microchimerism. This new finding may explain lack of successes in prenatal transplantation in hosts with intact immune system.

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AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION IN DE NOVO AML PATIENTS ≤ 60 YEARS WITH INTERMEDIATE RISK CYTOGENETICS

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401 de novo AML patients ≤ 60 years were included in the ongoing trial of the German SHG AML96 study group. Patients were stratified according to cytogenetic risk. After double induction therapy they received risk adapted and priority based post-remission therapy. 304 patients were treated within the largest group of intermediate cytogenetic risk. First priority for post-remission therapy was related allogeneic peripheral blood stem cell transplantation (PBSCT), second priority autologous PBSCT after one course of conventional post-remission chemotherapy and third priority two courses of conventional Ara-C containing chemotherapy. The autologous stem cell harvest obtained after the first course of conventional post-remission therapy was taken for transplantation. The conditioning regimen for the autologous PBSCT consisted of either TBI and cyclophosphamide or a combination of busulfan, VP-16 and cyclophosphamide. 71% of de novo AML patients ≤ 60 years with intermediate risk cytogenetics reached CR criteria after double induction therapy. These intermediate risk patients had favorable survival with autologous PBSCT. Median OS was 27 months for conventional chemotherapy as post-remission therapy, whereas the median survival for autologous PBSCT or allogeneic PBSCT was not reached yet. Finally, DFS after 54 months was 63% for autologous PBSCT compared to 59% for allogeneic PBSCT. DFS for conventional post-remission chemotherapy was as low as 40% after 32 months. In conclusion, autologous PBSCT for second post-remisson therapy is a well tolerated procedure with favorable outcome and lasting remissions in de novo AML patients ≤60 years with intermediate cytogentic risk.

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ADOPTIVE T-CELL THERAPY FOR PERSISTENT HCMV INFECTION

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Late HCMV disease remains a major concern after allo-SCT. Delayed HCMV-specific T-cell reconstitution and persistent HCMV infection have been described as major risk factors. Adoptive transfer of HCMV-specific T-cells was shown to restore protective immunity to HCMV. Thus, HCMV-infected patients not responding to antiviral chemotherapy for >4 weeks and lacking HCMV-specific T-cell responses were offered adoptive immunotherapy. T-cell lines were generated by repetitive stimulation of PBLs with HCMV-antigen. Following 3 specific restimulations the frequency of HCMV-specific T-cells (predominantly CD4+ T-cells) could be increased and alloreactive T-cells depleted in 14/20 donors. 6/14 patients did not receive T-cell therapy (clearance of HCMV-DNAemia, n=5; refractory acute GvHD, n=1). Thus, 8 patients received 10^7 /m^2 T-cells a median of 120 (79-479) days post transplant. At the time of T-cell therapy patients had received a median of 8 (4-10) weeks of antiviral chemotherapy and the median viral load determined by quantitative PCR was $1x10^4$ ($0.8x10^3$-10^5) genomes/ml blood. T-cell lines were transferred even in a patient receiving a graft from a 3 antigen-mismatched donor. Two weeks following transfer HCMV-specific T-cell proliferation was demonstrated in all 7 evaluable patients, in 5 of them ELISPOT and/or intracellular cytokine staining revealed 0.1-2.1% HCMV-specific CD4+ T-cells. In 2 HLA-A*0201 and 1 HLA-A*0101 positive patients tetrameric HLA/peptide complex and/or intracellular cytokine staining revealed 0.5-1% of CD8+ T-cells being HCMV-peptide-specific 30 days post-transfer. HCMV load significantly dropped in spite of cessation of antiviral chemotherapy in 7 evaluable patients, with maximal reduction of viral load after a median of 23 (14-42) days. In 5 patients HCMV infection was persistently controlled, in 2 patients with high viral load the antiviral effect was only transient. A decrease in telomer length of HCMV-specific T-cells, following ex-vivo expansion as demonstrated by Flow-FISH technology could be one reason for short term persistence of transferred T-cells.

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EVALUATION OF SAFETY, EFFICACY AND PHARMACOKINETICS OF ADMINISTERING TWICE DAILY IV BUSULFAN (BUSULFEX®) IN PATIENTS WITH ADVANCED HEMATOLOGIC MALIGNANCIES UNDERGOING STEM CELL TRANSPLANT (SCT)

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Intravenous busulfan(IVBU) has demonstrated safety and efficacy when administered at 0.8 mg/kg/dose IV Q6H x 16 doses. We evaluated the efficacy, safety and pharmacokinetics(PK) of giving the same total daily dose in a twice-daily schedule. Six patients with hematologic malignancies were treated. The preparative regimen consisted of IVBU 1.6mg/kg/dose over 4 hours Q12H x 4 days, followed by cyclophosphamide(CY) 60mg/kg IV QD x 2 days. Blood specimens were collected on 1st and 5th and 7th dose to determine the disposition of IVBU. Peripheral blood stem cells were given two days after completion of CY. G-CSF 5 mcg/kg was started on the day 0. GVHD prophylaxis consisted of tacrolimus plus methotrexate for allogeneic recipients. One patient developed a presumed fungal pneumonia and died of sepsis and MSOF on day +21. The remaining patients engrafted (ANC>500/μL) at a median of 10 days and sustained platelet counts>20,000/μL at 12 days. Significant RRT (grade III-IV) toxicity was limited to catheter infection(2), mucositis(1), hyperglycemia(1), pneumonia(1) and sepsis(1). Patient data and results are summarized below. First dose AUC was predictive of later doses. Despite higher AUCs, in the early post-transplant period there was no evidence of VOD, nor significant CNS, pulmonary or other end-organ toxicity. Conclusion: Twice daily dosing of IVBU is safe, efficacious and tolerable for patients undergoing hematopoietic SCT.

Patient	Diagnosis/ Transplant Type	Age	AUC (μM*min) 1st Dose	AUC (μM*min) 7th Dose	t 1/2 (hours)	Clearance (ml/min/kg)	Cmax (mcg/ml)	Total Bilirubin max	AST max	ALT max
1	NHL–Autologous	65	3418	3279*	3.64	1.89	2.10	0.7	41	37
2	AML–Allogeneic	60	4677	4580	3.41	1.40	2.56	2.6	37	23
3	NHL–Autologous	43	3108	3203	2.95	2.10	1.99	0.8	96	130
4	NHL–Autologous	49	4213	4560	3.10	1.55	2.45	1.9	59	52
5	NHL–Autologous	55	3375	3190	3.89	1.92	1.85	1.3	60	105
6	CML–Allogeneic	26	2661	2400	3.78	2.43	1.75	1.2	55	119
Median		52	3816	3203	3.53	1.91	2.05	1.3	57	79

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QUALITY OF LIFE (QOL) IN WOMEN WITH BREAST CANCER (BC) AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT)

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We conducted a prospective analysis of BC patients undergoing ABMT to assess how quickly and completely QOL is recovered. All women enrolling in three ABMT protocols (stages 2-4 BC) were invited to complete pre-validated, self-administered questionnaires (FAC-Br) at intake, discharge, and multiple points post-discharge. The questionnaire measured physical, social, emotional, functional, and general well-being, plus breast cancer-specific issues. At 1.5 years of accrual, 25 women were eligible; 1 refused, 7 expired during participation, and 1 withdrew. Nineteen had data for 3 months; 8 for one year. The mean age was 48, 62% were married, and 67% worked outside the home. For physical, functional, and general well-being, patients showed a nadir at discharge, with recovery beginning by thirty days, and back to baseline at 90 days (p=.05). For social and emotional well-being, no

change was observed from baseline through to one year. Comparison of general well-being by survival showed a lower baseline QOL for women who ultimately died, with a discharge nadir proportionate to their surviving counterparts but a swifter, more dramatic recovery (NS). Survival did not affect the lack of trend observed in social and emotional domains. Conclusions: The observed nadir in QOL at discharge in our sample is consistent with published reports, but subsequent recovery was even more rapidly complete than previously demonstrated. This may indicate that women undergoing ABMT for BC do not experience significant debilitation as has been suggested. The observed lack of variability in social/emotional domains also deviates from previous reports, and may reflect the intense family/social/medical support characteristic of our patient group. Poorer baseline QOL and amplified rate of recovery in patients who expired within one year suggest that these patients are not only sicker at outset, but may also be resistent to, or fast metabolizers of chemotherapy, explaining both diminished toxicity and decreased efficacy.

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REPERTOIRE ANALYSIS OF CD8+ AND CD4+ T CELL RESPONSES TO MINOR HISTOCOMPATIBILITY ANTIGENS INVOLVED IN THE DEVELOPMENT OF GRAFT-VERSUS-HOST DISEASE

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Graft-versus-host disease(GVHD) is a major complication of allogeneic hematopoietic-stem-cell transplantation. GVHD can be induced between MHC-matched murine strains expressing multiple minor-histocompatibility antigen(miHA) differences. Lethally irradiated C.B10-H2b/LiMcdJ(BALB.B) mice were transplanted with C57BL/6By(B6) T-cells and positively-selected miHA T-cell blasts were collected 5 days later from the thoracic-duct-lymphocyte(TDL) pool. T-cell receptor(TCR) CDR3-size-spectratyping analysis was used to examine the repertoire of the responding CD8+ and CD4+ TDL subsets at a single time point early in the development of GVHD. Spectratype analysis of the CD8+ TDL indicated that Vβ1,4,6,8-11 and 14 exhibited biased CDR3-size skewing. The CD4+ TDL exhibited biased CDR3-size skewing in the Vβ2,4 and 6-14 TCR families. Positively-selected TCR Vβ-skewed CD4+ and CD8+ T-cell subsets injected into irradiated BALB.B recipients were capable of inducing lethal GVHD. BALB.B mice transplanted with non-skewed Vβ CD4+ T-cells survived with minimal symptoms of GVHD. Given that GVHD is a dynamic process, we extended these studies to examine the T-cell repertoire responses during the course of disease. For this evaluation, lethally irradiated BALB.B recipients were transplanted with either host-presensitized B6 CD4+ or CD8+ T-cells. Spleens from transplanted mice were harvested at various time points and spectratyping was used to examine the TCR Vβ repertoire of the responding GVH-reactive T-cells. The results demonstrated only a few constant size skewings within any Vβ family throughout the course of GVHD. In the CD8+ responding T-cell population, Vβ5 and 16 exhibited the most consistent skewing at every time point examined. In the CD4+ T-cell population, Vβ3, 6, 10 and 11 demonstrated the most consistent skewing at every time point examined. The fluctuations of the CDR3-size skewing of the T-cell subsets could be a reflection of the increased processing, frequency and presentation of miHA as GVHD progresses in conjunction with the constant trafficking of T-cells through the splenic lymphoid compartment.

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HIGH DOSES OF CD34+ CELLS AND CHEMOMOBILIZATION ARE ASSOCIATED WITH A REDUCED PROGRESSION RATE AFTER AUTOLOGOUS PBSC TRANSPLANTATION FOR BREAST CANCER IN A PHASE III TRIAL

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We hypothesized that intensive chemotherapy would improve PBSC mobilization and disease control in a randomized controlled trial of 141 patients comparing results of chemomobilization with cisplatin 135 mg/m^2, etoposide 1200 mg/m^2, and cyclophosphamide 4.5 g/m^2 over 3 days plus G-CSF (6mcg/kg q12h) (CVP-G), with G-CSF alone with the collection goal >3x10^6 CD34+ cells/kg. Patients received cyclophosphamide 6 g/m^2, thiotepa 750 mg/m^2 and BCNU 450 mg/m^2 over 3 days with PBSC transplantation for treatment of breast cancer. Stratified by stage, 71 were randomized to the CVP-G group, 70 to G-CSF alone. 74 patients were high-risk stage II-III, 67 were stage IV. The CVP-G group had improved mobilization with a collection median of 18(range 3-141)x10^6 CD34+ cells/kg compared with 4(1-8)x10^6/kg for the G-CSF group. The median CD34+ dose infused was 11(2-89) vs 3.5(.7-7.2)x10^6/kg, respectively. Granulocyte and platelet recovery post-transplant was 9 and 9 days for the CVP-G mobilized group, 10 and 11 days for the G-CSF group (p<0.001). Breast cancer progression at 3 years was reduced in patients mobilized with CVP+G-CSF 34±6% vs. 59±7% for G-CSF alone (p=0.02). Progression was reduced in patients receiving CD34+ cell doses >7x10^6/kg (25±7% vs. 62±6%, p=0.0006), independent of the effects of CVP and disease stage. Among patients receiving CD34+ doses <7x10^6/kg, the progression rate was 61±12% in the CVP-G group and 62±7% in the G-CSF group. The CVP-G patients receiving CD34+ cell doses >7x10^6/kg had a progression rate of 24±7%; only one G-CSF patient received a dose >7x10^6/kg. For stage II-III patients, disease progression rates were 11±7% for CD34+ doses >7x10^6/kg vs. 60±9% for doses <7x10^6/kg (p=0.002), and 41±12% vs. 62±8% (p=0.08) respectively for stage IV. Use of CVP mobilizing chemotherapy and higher doses of CD34+ cells transplanted are associated with a lower risk of disease progression. The mechanism of this cell dose effect is uncertain.

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ADENOVIRUS INFECTIONS IN PEDIATRIC HEMATOPOIETIC STEM CELL TRANSPLANT (HSCT) RECIPIENTS: PROBABLE EFFECTIVENESS OF ANTIVIRAL THERAPY WITH CIDOFOVIR

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Adenovirus is a common, potentially life-threatening pathogen in HSCT patients. Effective antiviral therapy has not been clearly identified. Recent in vitro studies in our laboratory show that Cidofovir inhibits adenovirus growth at levels achievable in vivo. Between February 1994 and October 2000, post-transplant adenovirus infections occurred in 19 of 140 patients, including 17 of 77 allografted patients and 2 of 63 patients receiving autografts. Median patient age at HSCT was 6 years (range, 8m – 17y). Initial infection occurred at a median of 48 days post-transplant (range, day +7 - 1119). Sites of infection included the upper respiratory tract (N=9), lower respiratory tract (N=2), gut (N=15), bladder (N=5), and blood (N=1). Nine patients had multiple sites of infection. Autografted patients recovered without specific therapy. Prior to January 1999, 7 allografted patients developed adenoviral infections and were managed with supportive care alone. Five of these patients had lifethreatening infections (pneumonia[2], hemorrhagic gastroenteritis[2], disseminated[3]). In 3 cases, disseminated adenovirus infection was a cause of death. Since January 1999, patients with progressive adenoviral infections have been offered treatment with Cidofovir (5mg/kg IV q.week x 4, then q.2weeks x 2 and discontinued dependent on tolerance and symptomatic improvement). To date, 5 patients have received Cidofovir therapy, all for progressive gastroenteritis. Each patient experienced symptomatic improvement with Cidofovir; 5/5 had treatment-associated clearance of culturable virus from infected site(s). No serious Cidofovir toxicity occurred. An additional 5 allografted patients with milder adenoviral infections after January 1999 were managed without Cidofovir and recovered with supportive care. Viral isolates from 4 Cidofovir-treated patients were studied for in vitro sensitivity. Cidofovir concentrations inhibiting virus plaque formation by 50% (IC50) ranged from 0.6 – 2.3 micrograms/ml, levels consistent with clinical efficacy. Based upon these preliminary laboratory and clinical findings, Cidofovir mer-

its further investigation for adenovirus treatment in immunocompromised patients.

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NUCLEATED CELL DOSE IN UNRELATED CORD BLOOD UNITS CORRELATES WITH ENGRAFTMENT IN ADULT RECIPIENTS

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Nucleated cell dose (TNC) in unrelated cord blood (UCB) units may be a key feature in neutrophil engraftment, thus limiting applicability in larger adults. We have performed 21 UCB transplants in adult patients with acute myeloid leukemia (PIF n=1, CR1 n=2, REL1 n=3, CR2 n=1, advanced n=3), chronic myelogenous leukemia (CP n=2, AP n=2, BC n=2), myelodysplasia (RAEBT n=1), multiple myeloma (REL1 n=3), and NHL (REL2 n=1). Median age was 48 (range 20-59); median weight 70 kg (range 52-126 kg). Conditioning regimens: cyclophosphamide/TBI/ATG (n=15), melphalan/TBI/ATG (n=4), busulfan/cyclophosphamide (n=2). Graft-vs-host prophylaxis consisted of prednisone with either cyclosporin (n=13), or tacrolimus (n=8). Degree of HLA match was 6/6 in 4, 5/6 in 9, and 4/6 in 8. Median infused TNC/kg was 1.8 X 10^7 (range 0.4-5.3). Ten patients received ex-vivo expanded UCB (Aastrom Bioscience, Inc). Median time to ANC > 500/mm^3 was 30 days (Kaplan-Meier estimates) including 24% non-engraftment by day 60. Among recipients of UCB containing less than 2 X 10^7 TNC/kg the median time to ANC > 500/mm^3 was 35 days including all non-engrafting patients. By contrast, recipients of > 2 X 10^7 TNC/kg achieved engraftment at 18 days (p=0.126). This TNC/kg correlation is similar to adult Eurocord experience (>1.7 X 10^7 TNC/kg; ASH 2000) We found no evidence that HLA disparity adversely affected engraftment. Median time to platelets > 50,000/mm^3 was 56 days. Toxic deaths before day 30 occurred in 4 (19%) (MOF n=2, sepsis n=1, DAH n=1). Toxic death between day 31-100 occurred in 9 (43%) (GVHD n=2, sepsis n=6, adenovirus n=1, CNS hemorrhage n=1) with one late infectious death. (VZV at day 425). Our experience suggests that delayed engraftment remains an issue in adult recipients of UCB, especially when the TNC/kg is below 2 X 10^7. We have added prophylactic granulocyte transfusions to our protocol.

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PREEMPTIVE GANCICLOVIR AFTER ALLOGENEIC STEM CELL TRANSPLANTATION BASED ON THE DIGENE HYBRID CAPTURE® CMV DNA ASSAY

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Although cytomegalovirus (CMV) remains a major source of morbidity and mortality after allogeneic transplantation, a consensus for the optimal detection, surveillance, and intervention for CMV has not been reached. In January 99, our center changed its detection assay and interventional strategy from shell vial and universal prophylaxis, respectively, to a nucleic acid amplification method ("signal amplification") utilizing the Digene Hybrid Capture®(HC) Assay Version 2.0. The advantages of this qualitiative system, one of only 2 commercially available assays with FDA approval for CMV detection, include that it: 1) does not require viable cells, 2) is significantly more sensitive than the shell vial or antigenemia assay, approximating the sensitivity of PCR-based assays, 3) is less labor-intensive, and 4) allows rapid turnover time (5h). These features enabled the conversion to a preemptive ganciclovir strategy based on the initial positive Hybrid Capture assay obtained as part of weekly blood (culture) surveillance after engraftment through D+90. Of the 49 consecutive evaluable patients (pts) screened (13 MUD, 36 related): 2 of 15 (13%) pts who had both seronegative recipient (R) and donor (D) status (low risk group) had a positive assay post-transplant (2 sib); of the 34 pts with either seropositive R or D status (high risk group), 19 (56%) developed CMV HC positivity on one or more episodes (R/D +/+ 13/20; +/- 4/7; -/+ 2/7). One pt had documented CMV colitis in the setting of Grade 4 aGVHD; the remaining pts had viremia only without associated evidence for CMV infection. Using this assay as a trigger for pre-emptive ganciclovir spared 15/34 (44%) high risk pts the complications and costs (eg., neutropenia, thrombocytopenia, transfusions, growth factors) associated with anti-viral therapy in this population.

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HUMAN CYTOMEGALOVIRUS EFFICIENTLY INFECTS DENDRITIC CELLS AND SUPPRESSES DC FUNCTION AND CELL-MEDIATED IMMUNITY

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Human cytomegalovirus (HCMV) infection is one of the most important and life threatening infections in patients undergoing allogeneic stem cell transplantation (SCT). In addition, HCMV infection seems to induce severe immunosuppression and is often followed by other life-threatening infectious complications, e.g. invasive aspergillosis. To better understand the effects of HCMV on dendritic cells (DC), the most professional antigen presenting cells, DC of healthy HCMV sero-negative donors were infected with endothelotropic HCMV strains in vitro. Twenty-four hours after virus infection, the efficiency of infection was determined by immunocytochemical staining of immediate early antigen (IEA). In addition, the cytokines IL-1β, IL-6, IL-10, IL-15, IL-18 and TNF-α were analysed in the DC culture supernatant. Furthermore the expression of CD1a and CD83, MHC class I and II as well as the co-stimulating molecules CD80, CD86 and CD40 were determined on infected and uninfected DC by flow cytometry. For their immunostimulatory function, the DC were tested in a mixed lymphocyte reaction (MLR). As a negative control mock infected DC of each donor were cultured and analysed under the same conditions.

After 24 h of infection 60%-80% of the DC infected with endothelotropic HCMV strains expressed HCMV specific protein, e.g. IEA. Whereas the expression of CD1a, CD83 and MHC II was not significantly different between non-infected and infected DC, MHC class I, CD80 and CD40 were significantly reduced in surface expression as demonstrated by flow cytometry analysis. The proliferation rate with infected DC as stimulator cells in the MLR was 2-3 fold lower compared to the one with non infected DC as stimulator cells.

In conclusion, endothelotropic HCMV strains efficiently infect dendritic cells, reduce the expression of MHC class I and costimulatory molecules and thus suppresses cell-mediated immunity. This might be one explanation of HCMV mediated immunosuppression.

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RAPID SEQUENCE TANDEM STEM CELL TRANSPLANT FOR CHILDREN WITH HIGH-RISK NEUROBLASTOMA

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Autologous bone marrow transplantation has improved the event-free survival (EFS) of children with high risk neuroblastoma. However, the majority of these patients still relapse. To increase dose intensity during consolidation therapy, we have designed a Phase II trial for children with advanced neuroblastoma utilizing induction chemotherapy followed by tandem high-dose, myeloablative treatments with stem cell rescue (HDT/SCR) in rapid sequence. Patients initially received induction chemotherapy during which local control was completed and peripheral blood stem/progenitor cells collected. Patients then received tandem courses of HDT/SCR, 4 to 6 weeks apart. 55 patients are evaluable, median age 3 yrs, and 94 cycles of HDT/SCR have been completed to date. Pheresis has been possible for every patient, despite the young age of these patients, with an average of

7.2x10^6 CD34+ cells/kg available to support each HDT/SCR cycle and no differences in numbers of cells collected in younger or older patients. Patients experience rapid engraftment during both HDT/SCR cycles, with a median time to neutrophil engraftment of 11 days. Four patients who completed the first HDT course did not complete the second, and there were four toxic deaths. With a median follow-up of 22 months from diagnosis, 38 of 55 patients (3 year EFS 58%) remain event-free. A subset of the patients received stem cells purged by CD34 selection. The engraftment and EFS of these patients are similar to the overall group. This work demonstrates that a tandem HDT/SCR regimen for high-risk neuroblastoma is a feasible treatment strategy in children and may improve disease-free survival.

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SUCCESSFUL STEM CELL PURGING USING CD34 SELECTION IN PATIENTS WITH NEUROBLASTOMA

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CD34 selection for purging tumor from peripheral blood progenitor cells (PBPC) collected from patients with neuroblastoma (NB) has been questioned because of reports of detection of low levels of CD34 on the surface of some NB cells. We have used 3 approaches to address the issue of purging of NB from PBPC and possible labeling of NB. 1) FACS detection of CD34 on NB cell lines. We analyzed 6 NB lines for CD34 expression using the anti-CD34 MoAbs 9C5 (used in the Isolex system), 12.8 (CellPro) and QBend10 (CliniMACS), HPCA-1 and HPCA-2. Two of these lines were previously reported to express CD34. Most MoAb/cell line combinations showed no increase in labeling over control. Minimal labeling with 9C5 and QBend10 was eliminated after forward/side scatter gating to exclude debris. 2) Spiking experiments with the Isolex 50 system. PBPC were spiked with NB cells and the products purified using the Isolex 50. Purging of NB was assessed by quantitative multiplex RT-PCR (Taqman system) using primers to detect GAGE and normalizing to GAPDH showing depletion of NB (>2 logs). 3) Analysis of clinical specimens. PBPC pre and post CD34 selection were analyzed from patients treated on a tandem transplant trial. 30 PBPC aliquots were selected using the Ceprate LC CD34 selection system and analyzed using high-sensitivity NB immunocytochemistry (ICC). In 9/30 specimens where tumor was detectable, we again observed >2 logs of NB purging. We then analyzed 23 PBPC aliquots from products infused into tandem transplant patients, both pre and post CD34 selection, by RT-PCR. 20/23 specimens showed depletion of NB, although some level of GAGE message was observed in most post-CD34 selection specimens. These data show that purging of NB from PBPC specimens using CD34 selection is feasible, yielding infused products that are negative by ICC but positive by RT-PCR.

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MIROSTIPEN (CKβ-8, MPIF-1) PROTECTS LONG-TERM REPOPULATING BONE MARROW HEMATOPOIETIC STEM CELLS FROM 5-FLUOROURACIL (5-FU) INDUCED CYTOTOXICITY IN VIVO

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Mirostipen, a novel human β-chemokine, inhibits colony formation by human and mouse myeloid progenitors in vitro and protects these progenitors from cytotoxic drugs and irradiation in vivo. The present study examines the effect of mirostipen administration on the recovery of the long-term repopulating hematopoietic stem cell following 5-FU administration. Donor mice (C57BL/6-Ly 5.1) were administered with mirostipen or vehicle i.p. twice daily for 6 consecutive days. On days 3 and 6, these mice received i.v. bolus injection of 5-FU (100 mg/kg). Mice were then sacrificed on day 7 and bone marrow cells from each treatment group were pooled to assay for the presence of long-term repopulating stem cells. For transplantation, 10^5 bone marrow cells from each of the treatment groups were injected intravenously into sublethally irradiated (2 x 4.0 Gy) recipient mice (C57BL/6). After six months, bone marrow and spleen cells of the recipient mice were analyzed by flow cytometry to determine the percentage of donor derived myeloid and lymphoid cells. Irradiated recipient mice that received bone marrow cells from mice treated with mirostipen contained 4-fold higher donor derived cells in both spleen and bone marrow compared to mice that received bone marrow cells from the vehicle control ($p < 0.001$). Lineage analysis showed that bone marrow from mirostipen treated mice efficiently reconstituted both myeloid and T- and B-lymphoid compartments. These pre-clinical studies demonstrate that mirostipen administration results in the protection of long-term repopulating bone marrow hematopoietic stem cells, thereby making mirostipen an attractive drug candidate for evaluation in bone marrow autotransplant patients where the preservation of a functionally intact stem cell compartment is desirable.

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ASSOCIATION OF BRONCHIOLITIS OBLITERANS ORGANIZING PNEUMONIA (BOOP) WITH ACUTE AND CHRONIC GRAFT-VERSUS-HOST DISEASE

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Fifty-one patients with histologic bronchiolitis obliterans organizing pneumonia (BOOP) were identified by review of all open-lung biopsies (n=817) from hematopoietic stem cell transplant patients reported over a 22 year period. Two cases were subsequently excluded because they occurred following autologous stem cell transplantation. The mean age of the remaining patients was 30.1, and 78% were male. The primary disease was ANL in 41%, ALL in 22%, CML in 22%, lymphoma in 6%, MDS in 6%, and other diagnoses in 3%. Onset at a median of day 108 after transplantation was with fever (61%), dyspnea (45%), non-productive cough (43%) and crackles (48%). BOOP remained stable or resolved in 78% of patients. It progressed in 22%, and most of these patients died of respiratory failure. The study design did not allow assessment of whether corticosteroid therapy was beneficial. Microorganisms isolated from 7 of the cases included CMV alone (3), CMV and parainfluenza 3 (1), RSV (2), and Candida parapsilosis (1). Four allotransplant control subjects were matched to each case according to year of transplant and age at transplant. Case and control subjects differed in primary disease diagnosis and pretransplant conditioning regimen. They did not differ in gender, underlying disease activity, HLA matching with donor, GVHD prophylaxis, or recipient or donor CMV antibody status. Histological BOOP was associated with both acute (P=0.001) and chronic (P=0.036) GVHD. By multivariate analysis, the odds ratio (adjusted for other variables associated with BOOP) for acute GVHD was 3.21 (95% CI, 1.15 to 8.99) and for chronic GVHD was 8.68 (95% CI, 1.81 to 41.62). When the conditional logistic regression model was adjusted for the presence of both acute and chronic GVHD, no other variable was associated with BOOP. Patients with BOOP were more likely to have acute GVHD involving the skin (P=0.003) and liver (P=0.007).

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HIGH DOSE MELPHALAN(HDM) AND TOTAL BODY IRRADIATION(TBI) WITH PERIPHERAL STEM CELL TRANSPLANTATION(PSCT) FOLLOWED BY INTERFERON (IFN) FOR MULTIPLE MYELOMA

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Fifty-nine patients with the established diagnosis of multiple myeloma (MM) received HDM 140 mg/m^2 with TBI(1200 cGy) or HDM 200 mg/m^2 if they had prior radiation. The fractionation of the TBI was modified early in the study due to a high incidence of pulmonary toxicity. All patients were mobilized with cyclophosphamide at 4 gm/m^2 and G-CSF and were to receive IFN post transplant. Median age was 54 years (38 - 71 years). The median number of prior chemotherapy regimens is 2 (range 1- 3). Fourteen of 40 patients evaluable for IFN therapy received IFN for minimum of 6 months. The reasons for not receiving interferon

are varied and will be presented. OS and RFS was 51.3% and 18.6% at 65.5 months with a median follow-up of 12.1 months. Treatment related mortality was 3.3%. There was a significant improvement in overall survival (OS) in patients who received IFN (p=0.009) and a trend to improved relapse free survival (RFS) (p=0.07) Treatment with TBI predicted for better RFS and OS. Those receiving TBI had 25.7% RFS and 58.7% OS at 65 months vs. 0% RFS and 35.2% OS at 31 months in those not receiving TBI (p = 0.004). Those patients not receiving TBI may have been a poorly selected group, with most having received prior radiation. As well, patient selection may play a role in the IFN results, with better results in the patients who tolerated the IFN. Abnormal cytogenetics prior to PSCT were found in three patients(8%). All 3 relapsed and died by 30 months. In comparison, 53% of those with normal cytogenetics were relapse-free at 30 months of follow-up. Abnormal cytogenetics are a poor predictive factor and alternative therapy such as allogeneic transplants should be considered in this high risk group.

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EARLY PREDICTION OF POOR HEMATOPOIETIC PROGENITOR CELL (HPC) MOBILIZATION DURING APHERESIS

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Failure of HPC mobilization (MOB) wastes resources and causes clinical delay. Records of 64 consecutive autologous HPC patients (PTS) were analyzed to determine whether poor MOB could be identified early enough to allow salvage interventions (e.g. altered cytokine dose, changes in apheresis parameters). Diagnoses included breast cancer (22), NHL (20), myeloma (11), Hodgkins (5), CML (5), amyloid (2), CLL (1). Two MOB regimens were used: 1) cyclophosphamide (CTX) 3 gm/m^2 and G-CSF 10 mcg/kg/d followed by apheresis when WBC >1000/mm^3 or 2) G-CSF 10 mcg/kg/d for 4 days followed by apheresis. Apheresis collections were performed for 5 days or until a target of >4.0x10^6 CD34+ cells/kg was reached. G-CSF was given prior to each apheresis. RESULTS with CTX/G-CSF (33 PTS): 25 PTS reached target after 1 or 2 collections (76%). 3 PTS had >1.0x10^6/kg after 2 collections and all reached target after 5 collections. 5 PTS had <1.0x10^6/kg after 2 collections, and only 1 reached target after 5 collections (p=0.0001). RESULTS with G-CSF alone (31 PTS): 8 PTS reached target after 1 or 2 collections (27%). 15 PTS had >1.0x10^6/kg after 2 collections but only 7 reached target after 5 collections, all 7 having >1.5x10^6/kg. 8 PTS had <1.0x10^6/kg after 2 collections, and none reached target after 5 collections (p=0.002). ANALYSIS: Using <1.0x10^6 CD34+ cells/kg after 2 days to define poor MOB, 15% (5/33) of CTX/G-CSF PTS and 26% (8/31) of G-CSF only PTS failed to mobilize HPC. Only 1 of these 13 combined poor MOB PTS reached target after 5 aphereses (p<0.0001). Subset analysis of G-CSF only PTS using <1.5x10^6 CD34+ cells/kg to define poor MOB was also highly predictive (p<0.0001). CONCLUSION: Interventions to salvage MOB seem indicated for patients failing to achieve >1.0x10^6 CD34+ cells/kg after 2 aphereses following CTX/G-CSF, or >1.5x10^6 CD34+ cells/kg following G-CSF alone.

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QUANTITATION OF T CELL NEOGENESIS IN VIVO AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION IN ADULTS

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Following myeloablative therapy, it is unknown to what extent age-dependent thymic involution limits the generation of new T cells with a diverse repertoire. Normal T cell receptor (TCR) gene rearrangement in T cell progenitors results in the generation of T cell receptor rearrangement excision circles (TREC). TREC are stable episomal DNA circles which are not replicated and are therefore diluted with subsequent cellular divisions. In this study we used a quantitative assay for TREC to measure T cell neogenesis in 18 adult patients with CML who received myeloablative therapy followed by transplantation of T cell depleted allogeneic hematopoietic stem cells. These patients offered an opportunity to determine the kinetics of immune reconstitution from normal hematopoietic stem cells in a setting devoid of additional immunosuppression. Although phenotypically mature T cells had recovered by 1-2 months post BMT, TREC levels remained low for 3 months after BMT. T cell neogenesis became evident by 6 months and normal levels of adult thymic function were restored at 6-12 months post-BMT. Subsequent leukemia relapse in 13 patients was associated with reduced TREC levels but infusion of mature donor CD4+ T cells resulted in rapid increase of TREC levels indicating restoration of thymic function. The return of normal levels of TREC in patients after myeloablative therapy demonstrates that T cell neogenesis contributes to immune reconstitution in adult patients. The low levels of TREC detected at relapse suggest that relapse of leukemia markedly affects the ability to maintain normal thymic function and normal levels of T cell neogenesis. The rise in TREC levels after DLI implies that thymic function in adults is not static and can be manipulated in vivo. The quantitative measurement of TREC offers a new method for assessment of T cell neogenesis and the effects of different treatments on thymic function in vivo.

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PERIPHERAL BLOOD PROGENITOR CELL (PBPC) PREPARATIONS EXPRESS TISSUE FACTOR (TF) ANTIGEN AND ACTIVITY

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Patients undergoing peripheral blood progenitor cell (PBPC) transplantation can develop laboratory and clinical evidence of a coagulopathy. We hypothesized that PBPC contain procoagulant activity that contributes to the deleterious effects of transplantation, including coagulopathy and venoocclusive disease. We assessed TF antigen and activity in PBPC collected for autologous transplantation. Patients received Cytoxan (2 or 4 g/m^2) on day 1, VP-16 (200 mg/m^2) on days 1-3, then G-CSF (10 μg/kg) daily from day 4 until collection of PBPC was completed. Most underwent three or more apheresis procedures. Samples of the PBPC products were analyzed by flow cytometry (12 samples, 8 patients) and TF activity assays (8 samples, 6 patients). The first collection from each patient was sampled, as were additional collections from 3 patients. Fluorochrome tagged antibodies to TF and surface antigens were used to determine the proportion of TF+ cells and correlate TF and lineage specific marker expression. The number of TF + cells ranged from 0.2% to 11%. Neither CD34+ cells, nor lymphoid cells expressed TF antigen. A subpopulation of myeloid (CD33+) cells were positive for TF, with most of TF+ cells also expressing granulocytic markers (CD11b, CD15 and lactoferrin). However, the TF+ cells did not have the light scattering characteristics of mature granulocytes. They were larger and of lower granularity than granulocytes. None of the samples with less than 1% TF+ cells (3 samples, all 2nd collections) had coagulant activity in a prothrombin time-type assay. However, not every sample with higher proportions of TF+ cells had procoagulant activity. Procoagulant activity was due to TF since an inhibitory anti-TF antibody abolished clot formation. Thus, PBPC preparations, especially the first collection from a patient, contain myeloid precursors that express variable amounts of TF antigen and activity. This procoagulant activity may contribute to complications of PBPC transplantation.

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NON-ADHERENT MARROW CELLS GIVE RISE TO THE MARROW STROMAL MICROENVIRONMENT AND MESENCHYMAL TISSUES AFTER BMT IN ANIMAL MODELS—TOWARD A MESENCHYMAL STEM CELL

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Although it is well established that hematopoietic stem cells can be transplanted, it remains unclear whether the same holds

true for the hematopoietic microenvironment. Recently, investigators have postulated the existence of a mesenchymal stem cell whose identity many consider to be an adherent marrow stromal cell. We specifically tested this hypothesis by studying the fate of gene-marked adherent and non-adherent marrow cells after BMT. Murine adherent marrow stromal cells were isolated and transduced with a retroviral vector encoding the green fluorescent protein (GFP). Gene transfer efficiency was >85%, as determined by flow cytometry for GFP. Transduced cells were infused intravenously after either lethal (1100 cGy) or sub-lethal (400 cGy) irradiation. Lethally irradiated mice were additionally given non-transduced, non-adherent marrow (hematopoietic) cells for hematologic rescue. At 1 and 3 months after infusion, mice were sacrificed and the adherent marrow cells were isolated. Flow cytometric analysis and PCR failed to demonstrate GFP+ cells. In separate experiments, non-adherent marrow cells were transduced with the same retroviral vector and FACS-sorted cells (>95% GFP+) or unsorted cells (~40% GFP+) were infused into lethally irradiated recipients. After 6 months, recipient mice were sacrificed and adherent marrow cells isolated. In contrast to our previous results, fluorescence microscopy unequivocally demonstrated GFP+ marrow stromal cells. Flow cytometry confirmed that up to 80% of recipient stromal cells were GFP+ and lacked hematopoietic cell antigen expression. Moreover, osteoblasts, adipocytes, and muscle satellite cells also expressed GFP. Finally, GFP+ marrow stromal cells (18%) were observed from a macaque rhesus monkey that received an autologous BMT with CD34+ PBSCs transduced with the GFP vector. Together, these results suggest the existence of a mesenchymal stem cell which resides within the non-adherent marrow compartment. Ongoing studies are aimed at determining whether there is a common ontogeny of hematopoietic and mesenchymal tissues.

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MISMATCHED BMT AFTER NON-MYELOABLATIVE CONDITIONING

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Most non-myeloablative transplants use HLA-matched, G-CSF–primed PBSC. We performed 3 HLA-mismatched unmanipulated BMT's after non-myeloablative conditioning with rapid and complete donor engraftment. The patients had refractory NHL, ALL and AML. One donor was sex-matched sibling mismatched at A-locus. One VUD was sex-matched (female) DRβ1-locus mismatched with 9 qh+ chromosome polymorphism. The other VUD was sex-mismatched (male) and A-locus mismatched. Conditioning was TBI 200 cGy, ATG, CSA and MMF. Grafts contained 6.42, 7.34 and 8.64[8] TNC and 0.63, 4.68 and 5.52 CD34+ cells/kg. CSA and MMF were continued post-transplant. Donor chimerism was assessed by cytogenetics,FISH and molecular HLA typing. ANC 500 occurred on d+7 and +11(VUDs) and +11 (related). PLT >20,000/μL was not reached by the related patient (expired d+29). VUD recipients achieved PLT >20,000/μL d+17 and +18. In all cases 100% donor chimerism was documented. By d+30, 2 patients with leukemia had NED by microscopy and flow cytometry and the NHL patient had reduction of bulky adenopathy. All three patients expired with multiorgan failure and evidence of GvHD of the liver. Graft rejection was not seen in this small group of recipients with extensive previous chemotherapy. Marked response to the transplant was seen in these patients who had refractory disease. Severe GvHD occurred in all patients. Although not unexpected, the presence of persistent malignant disease or normal host hematopoiesis may contribute to GvHD by displaying class II antigens. Mismatched stem cell transplantation can be accomplished across HLA barriers using non-myeloablative conditioning. GvHD rather than rejection appears as a limiting factor to the success of this transplants. Further refinements in conditioning and/or graft manipulation may result in improved outcomes.

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AN INTERIM LOOK: NON-MYELOABLATIVE CHEMOTHERAPY AND ALLOGENEIC STEM CELL TRANSPLANT (ALLOSCT) FOR MULTIPLE MYELOMA IS SAFE AND EFFECTIVE

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Multiple myeloma (MM) is often treated with autologous stem cell transplant (AutoSCT), offering higher complete remission rates and longer survival than conventional chemotherapy. Most patients eventually relapse. AlloSCT is potentially curative (graft versus myeloma effect) but is associated with unacceptable mortality. Non-Myeloablative AlloSCT seems to be a reasonable option for myeloma patients. 4 patients with MM (IgG kappa, IgG lambda, IgD Lambda, and kappa-light chain) and 1 with multiple plasmacytoma were treated. There were 2 males and 3 females. Two of the five had relapsed after prior AutoSCT. The non-myeloablative transplant regimen included Fludarabine (30mg/m^2 x 5d), Melphalan (140mg/m^2) and Anti-thymocyte globulin (ATG at 10mg/kg x 4d: days -3, -1, +1, and +3). AlloSCT followed with Cellcept (weeks 2, 3, & 4) and Cyclosporine being used for graft versus host disease (GVHD) prophylaxis. Patients received a mean of 8.81 x 10^6 CD34+ cells/kg and 3.60 x 10^8 CD3+ cells. Mean time to neutrophil engraftment (ANC 500/μL) was day 18 (range day 6 to 50). Mean time to platelet engraftment (>20,000/μL) was day 42 (range day 14 to 78). Clinically significant acute GVHD (Grade II) occurred in one patient. Three others had grade 2 skin GVHD. Extensive chronic GVHD occurred in 2/3 evaluable patients. Mean follow-up is 8.5 months and 4 of 5 patients are alive without disease progression. One patient died from myocardial infarction secondary to co-morbid conditions. This patient had IgD MM that had relapsed within 6 months of a prior AutoSCT. At the time of death, the patient was in remission with 100% donor engraftment. 3/3 evaluable patients reached full donor chimerism by the day 45-60 VNTR analysis and had disappearance of their monoclonal proteins. The above regimen seems to be well tolerated and effective. We plan to continue accrual.

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FASL AND PERFORIN CYTOTOXICALLY IMPARED DONOR T-CELLS INDUCE GVHD IN TNF RECEPTOR 1 (P55) KNOCKOUT MHC I/II MISMATCHED RECIPIENTS

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To understand the involvement of donor T-cell cytotoxic function in GVHD induction following allogeneic BMT, we examined GVHD development in the absence of three major cytotoxic pathways: perforin, FasL and TNF receptor 1 (TNF-R1). In a MHC I/II mismatched BMT model (H-2^b → H-2^d), 5•10^6 T-cell depleted donor bone marrow cells and varying numbers of T cells from FasL/perforin double deficient B6 donors (B6-cdd) were transplanted into MHC mismatched recipients 24 hours after lethal single dose TBI (8.5Gy). Compared to B6 wild type donor T cells, we previously reported that 10-fold greater numbers of B6-cdd T cells are required to induce a similar kinetic pattern of GVHD onset (e.g. 2.5•10^6 B6-cdd vs. 2.5•10^5 B6-wt). In the present studies, both TNF-R1 wild type and TNF-R1 deficient recipients developed lethal GVHD after transplantation of B6-cdd donor T-cells. The onset of recipients' weight loss indicated that both wild type and TNF-R1 deficient hosts developed GVHD with the same kinetic pattern when 5•10^6 or 1•10^6 T-cells from B6-cdd donors were injected. In addition, groups of recipients exhibited clinical signs of acute GVHD, including diarrhea, skin lesion, ruffled fur and haunched posture. Therefore, TNF-R1 deficiency did not protect recipients from skin and intestinal damage. Finally, no significant difference between mean survival times (MST) of wild type and TNF-R1 deficient recipients receiving B6-cdd donor T-cells was observed. In total, these results demonstrate that TNF-R1 is not the primary pathway for GVHD induction by T cells which lack the capacity to effect donor-anti-host cytotoxicity via perforin and FasL dependent signaling. Thus, we conclude that GVHD can still occur in the simultaneous absence

of perforin, FasL and TNF-R1 dependent cytotoxic function in this model.

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DIFFERING NUMBERS OF CYTOTOXICALLY IMPAIRED CD4+ DONOR T-CELLS IN RECIPIENT LYMPH NODES SUGGEST THAT FAS LIGAND FUNCTION IS CRUCIAL PRIOR TO GVHD PATHOGENESIS IN MHC CLASS I/II MISMATCHED BMT MODEL

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A number of allogeneic BMT models involving donor T-cells with cytotoxic deficiencies (perforin/granzyme and/or Fas ligand) have been examined to investigate the roles of donor T-cell cytotoxicity in GVHD. While these studies clearly demonstrated both FasL and perforin/granzyme dependent effector mechanism contribute to GVHD pathology, it remains unclear when and how following transplant, each of these cytotoxic pathways are important. In this study, we compared the GVHD induction efficacy of CD4+ T-cells from cytotoxic single (B6-gld *fasl*$^{gld/gld}$ or B6-pko *Pfp*$^{-/-}$) and double deficient (B6-cdd, *Fasl*$^{gld/gld}$*Pfp*$^{-/-}$), with wild type (B6-wt) donors (B6) in a MHC I/II fully mismatched BMT (8.5Gy) model. The combined absence of perforin and FasL function in B6-cdd CD4+ T-cells did not prevent induction of GVHD associated body weight loss, clinical changes and mortality. However, recipients of B6-cdd T-cells clearly showed a delayed disease onset. Examination of donor cell numbers early post-BMT revealed that the absence of perforin and fasL function resulted in a marked decrease in B6-cdd donor CD4+ T-cell numbers vs. B6-wt CD4+ numbers, which correlated with the former population's delayed ability to induce GVHD. Increasing the dose of TBI conditioning abolished this difference as well as the delayed induction of GVHD by B6-cdd CD4+ T-cells in the host, suggesting a role of host resistance. To determine whether perforin and/or FasL dependent cytotoxic function was responsible for these observations, individual cytotoxic single deficient and double deficient CD4+ populations were transplanted. These studies demonstrated that donor CD4+ T-cells in host LN is dependent primarily on FasL function. Additionally, rapid weight loss and 100% lethality occurred in recipients of all four CD4+ donor T cell populations. Experiments in progress focus on the early mechanism by which donor CD4+ T-cells overcome the host resistance and mediate GVH response in the host.

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SUPPRESSION OF ALLOREACTIVITY IN VIVO BY DONOR-DERIVED REGULATORY T CELLS MAY INVOLVE IL-10 AND FAS/FASL INTERACTIONS BUT NOT IL-4 OR PERFORIN

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We previously demonstrated that donor marrow-derived immunoregulatory T cells emerging from the host thymus suppress donor-anti-host alloreactivity induced by donor leukocyte infusion (DLI) after BMT. The dominant population of regulatory T cells were αβTCR+, Thy1+ and CD4+. Preliminary results indicated that the regulatory T cells could be adoptively transferred to suppress alloreactivity in recipients depleted of regulatory cells. We have investigated the mechanism of suppression induced by these regulatory T cells. Lethally irradiated AKR mice (H-2^k, Thy1.1+) were given TCD BM from C57BL/6 donors (H-2^b, Thy1.2+) and infused 28 days later with spleen cells from Thy1-congenic donors (H-2^b, Thy1.1+) to simulate DLI therapy. C57BL/6 mice deficient in FasL, perforin, IL-4 or IL-10 were used as BM donors to examine the role of these molecules in regulatory T cell-mediated suppression of alloreactivity. Recipients of marrow from IL-4 knockout or perforin knockout mice did not develop acute GVHD after DLI, suggesting that these molecules were not involved in regulatory cell activity. In contrast, recipients of IL-10 knockout BM had a significantly higher rate of GVH-related mortality than the DLI controls, indicating that IL-10 may be involved in the suppression of alloreactivity by regulatory T cells. Similarly, recipients of FasL knockout BM had more severe GVHD than chimeras given normal BM and DLI, suggesting that Fas:FasL interactions were also involved in suppression of alloreactivity by regulatory cells. Notably, BM chimeras given DLI with Fas-deficient spleen cells did not develop GVHD after DLI, indicating that FasL expressed on regulatory cells is involved in the suppression of alloreactivity by a mechanism other than inducing apoptosis of the infused donor T cells. Experiments are in progress to determine whether other immunosuppressive molecules, such as TGF-β, might also be involved in suppression of alloreactivity by donor regulatory T cells.

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PREDICTIVE FACTORS FOR UNSUCCESSFUL MOBILIZATION OF PERIPHERAL BLOOD STEM CELLS (PBSC) IN PATIENTS WITH NON-HODGKIN'S LYMPHOMA (NHL) AND HODGKIN'S LYMPHOMA (HD)

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Introduction: PBSC transplant has become increasingly utilized in patients with high risk NHL and HD. Factors have been identified which are associated with poor mobilization; however, their importance remains unclear.

Patients and Methods: Between 10/97 and 6/00, 111 patients were identified who underwent PBSC mobilization for NHL(90) or HD(21). NHL histology was low grade(n=10), intermediate grade(n=45), transformed(n=23) or other(n=12). The median age at mobilization was 53.2 years (range 12.4–76.1); 64 male:47 female. Stage was early(I/II) in 42 and advanced(III/IV) in 69. The median interval from diagnosis to mobilization was 18.4 months (range 3.6–342.4). The median number of prior treatment episodes was 3(range 1-6)and prior treatment cycles was 9(range 3-25). Bone marrow was positive at mobilization in 12. PBSC mobilization was with G-CSF(10 mcg/kg/day) in the majority of patients. The mobilization was considered successful if $\geq 2.0 \times 10^6$ CD34+ cells/kg were collected in three or less leukaphereses.

Results: 55 patients(49.5%) had a successful PBSC mobilization. Unsuccessful mobilization was significantly associated with female gender(p=0.04, odds ratio[OR] 0.45), increasing number of prior treatment regimens(p=0.04, OR=0.69) and increasing number of prior therapy cycles(p=0.02, OR=0.87). Mobilization success was not significantly associated with specific types of therapy. The only potential exception was the use of purine analogs, where none of the 9 patients who received this treatment had a successful mobilization. In a multivariate logistic model for mobilization success, gender and the number of prior therapy cycles were the two significant factors (p=0.035 and p=0.025, respectively).

Conclusion: Female gender and increased number of prior treatment cycles are significantly associated with unsuccessful mobilization in both univariate and multivariate analyses. These findings help to better define a population of patients with Hodgkin's and NHLs in which alternative regimens for stem cell collection should be developed.

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FOCUSING ON A LIMITED NUMBER OF T-CELL SPECIFICITIES AS POTENT INDUCERS OF GRAFT-VERSUS-HOST DISEASE ACROSS A MULTIPLE MINOR HISTOCOMPATABILITY ANTIGEN BARRIER

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CXBE recombinant inbred mice express a subset of the miHA repertoire expressed by BALB.B mice. As lethally irradiated recipients of naïve H2b-matched C57Bl/6 (B6) T-cells, both strains succumb to acute GVHD. However, purified B6 CD4+ T-cells can mediate severe GVHD only in BALB.B recipients, whereas CXBE recipients develop a much milder form of disease. CDR3-size spectratype analysis indicated a polyclonal, largely overlapping T-cell response in both strains, as well as evidence for specificities directed against unique BALB.B miHA. Histological analysis of GVHD target organs from both recipients revealed

similar amounts of infiltration and injury in the lingual epithelium, however significant differences were found in the crypt epithelium of the small intestine. This contrasting pathology suggested qualitative, rather than purely quantitative differences in the B6 CD4+ T-cell response between the two recipient strains. To investigate this notion, we transferred carboxy-fluorescein diacetate succinimidyl ester (CFSE) labeled B6 T-cells into both lethally irradiated recipient strains, and 4-5 days later analyzed the size of the responding B6 CD4+ T-cell pool. The data revealed that a similar percentage of labeled B6 CD4+ T-cells had undergone antigen driven division in both recipients, relative to a syngeneic B6->B6 control. To further study the pathological significance of the B6->BALB.B response, B6 CD4+ T-cells utilizing Vbeta families found involved only in the BALB.B response (Vbeta's 2 and 11) were selectively transferred into lethally irradiated BALB.B recipients. Both the Vbeta 2 and 11 only and whole CD4 groups demonstrated similar disease severity until at least 30 days post-transplant. Significantly, animals that received Vbeta 2 and 11 depleted CD4+ T-cells experienced slightly less severe GVHD than the other two groups. This suggests that Vbeta 2 and/or 11+ CD4+ T-cells may be contributing to the more severe GVHD observed in the BALB.B recipients.

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CHANGES IN LYMPHOCYTE SUBPOPULATIONS AFTER AUTOLOGOUS PBSCT IN CHILDREN TREATED WITH LOW-DOSE IL-2

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Interleukin-2(IL-2) exerts anti-cancer effect when the tumor burden is low. During immune reconstitution after stem cell transplantation, there is a persistent CD4 depletion. To see the effects of IL-2 on immune reconstitution in children, 14 patients (9 neuroblastoma, 4 AML, 1 Ewing sarcoma, age 6.1±4.2yr) were enrolled in this study from 1997 to 2000. IL-2 (Proleukin ®, Chiron) was started subcutaneously after neutrophil engraftment with the dose of 5MU/sqm for the first 2 days, 1MU/sqm for the subsequent 12 days, then followed by 14 days of rest. IL-2 was repeated more than 6 cycles. The CBC, total eosinophil count(TEC) and T lymphocyte subsets were checked before and after IL-2 therapy. The interval from PBSCT to start IL-2 was 46.7±36.3days. The follow-up duration was 15.2±7.7(2-26) months. Two of neuroblastoma patients relapsed 4, 11 months after PBSCT, respectively. A total of 95 cycles of IL-2 therapy was done without dose modification. All parameters of T-lymphocyte subsets increased after IL-2(see table). After the first cycle of IL-2 therapy, CD4/CD8 ratio decreased but subsequent IL-2 therapy resulted in increase of CD4/CD8 ratio and remained <1 for months. Low-dose IL-2 is well tolerated in all patients and there was marked increase in lymphocyte subpopulations. CD8+ increased prominently for the first 2 cycles, however CD4/CD8 ratio increased gradually thereafter. NK cell increased after each IL-2 therapy and sustained above normal range during follow-up period. Taken together, IL-2 would have some anti-cancer effects on early post-transplant period.

	(1) Before IL−2	(1) After IL−2	(2) Before IL−2	(2) After IL−2	(3) Before IL−2	(3) After IL−2	(4) Before IL−2	(4) After IL−2
TEC	236 ± 612	2736±6059	477 ± 744	4289±1804	428 ± 474	1351±1814	574 ± 793	2240±2467
$CD4^+$	215 ± 125	359±245	274 ± 281	381 ± 205	287 ± 128	465 ± 207	413 ± 186	637±329
$CD8^+$	770 ± 1073	1720±1958	861 ± 1089	1513±1650	852 ± 694	1008±1022	1086 ± 566	1210±864
NK	380 ± 607	742±831	421 ± 467	716 ± 402	505 ± 407	687 ± 377	565 ± 521	776 ± 629

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NOVEL APPROACHES FOR THE GENERATION OF LEUKEMIA-REACTIVE ALLOCYTOTOXIC DONOR T CELLS FOR PATIENTS WITH RELAPSED ALL POST ALLOGENEIC MARROW TRANSPLANTATION.

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We have evaluated the capacity of host-derived EBV-transformed B-lymphoblastic cell lines (BLCL), which express critical costimulatory molecules, to stimulate the generation of cytotoxic T cells against minor or major alloantigens expressed on host pre-B ALL cells. Matched-unrelated (HLA-A, B, DR and DQ) donor PBMC were stimulated weekly for 5 weeks with EBV BLCL derived from PBMC obtained from the patient with pre-B ALL during remission. Effector T cells tested in ^{51}Cr-release assay showed 39% and 28% lysis of the patient EBV BLCL and patient B-ALL cells and 9% lysis of third party B-ALL cells. In a second case haploidentical maternal T lymphocytes were stimulated with EBV BLCL, derived from the patient during remission and their killing capacity determined in functional assays. ^{51}Cr-release assay showed 30% lysis of both the patient's EBV BLCL and B-ALL cells, 15% lysis of patient PHA-blasts and 5% of third party B-ALL. In limiting dilution analysis we obtained a CTLp frequency of 1/633 against patient EBV BLCL, 1/10,253 against patient leukemia cells, 1/319,903 and 1/469,928 against patient PHA-blasts and third party B-ALL, respectively. Intracellular cytokine staining of IFN-γ– producing donor $CD8^+$ cells displayed 18% of the donor $CD8^+$ cells producing IFN-γ in response to the EBV BLCL, 4.8% in response to the patient's leukemia cells and only 0.5% and 0.1% following activation with PHA-blasts and third party B-ALL cells, respectively. These studies suggest that EBV transformed BLCL express alloantigens that are also displayed on patient B-ALL cells which can be targeted by sensitized HLA matched-unrelated or HLA disparate alloresponsive T cells. Such cells, if modified to express a drug sensitive gene, such as herpes simplex virus-thymidine kinase, could provide a more potent and controlled population of effector cells for adoptive therapy of acute lymphoblastic leukemia.

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$CD8^+$ BARRIER T-CELLS ELICIT INTACT BARRIER ACTIVITY AGAINST ALLOGENEIC MARROW TRANSPLANTS IN THE SIMULTANEOUS ABSENCE OF PERFORIN, FAS-L AND TNFR1 OR R2 SIGNALING.

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Engraftment failure following allogeneic bone marrow transplantation is of clinical concern using T-cell depleted innoculum (BMT-TCD), particularly in aplastic anemia recipients. Immune resistance by lymphoid and NK populations with "barrier" function is well established. We previously demonstrated that host $TCR^+CD8^+NK1.1^-$ cells effectively resist MHC-matched marrow allografts in B6 wild type (wt) recipients primed to donor BALB.B MiHA. Immunodominant CTL defined donor antigens appear to dominate the specificity of this allograft resistance, consistent with a response by MiHA-reactive $CD8^+$ T-cells. Host $CD4^+$ cells do not function as effector cells in the resistance observed, but are required for the generation of resistance activity, presumably supporting the expansion of $TCR^+CD8^+NK1.1^-$ barrier cells. T-cell lines generated from the B6-wt Spl and LN cells primed to BALB.B MiHA were exclusively $TCR\alpha\beta^+CD8^+NK1.1^-Mac1^-$ cells. Transfer of these cells into naive B6 recipients conferred resistance to BALB.B marrow allografts. Importantly, studies using the cytotoxically double (perforin/FasL) deficient (cdd) B6 mice primed to donor MiHA revealed that B6-cdd $CD8^+$ cells are capable of highly efficient allograft resistance. Barrier activity in B6-cdd against progenitor cells from $TNFR1^{-/-}$ or $TNFR2^{-/-}$ donors demonstrated resistance in the simultaneous absence of three major effector pathways. These findings indicate the involvement of alternative effector pathway(s), which could include ligands of other TNF-family members or non-cytotoxic inhibitors (i.e. TGF-β) in resistance. Notably, MiHA reactive T-cell lines recently generated from B6-cdd were predominantly $TCR^+CD8^+NK1.1^-Mac1^-$ cells, and also contained a high percentage of Vβ5 TCR^+ cells, consistent with a limited response against selected (i.e. immunodominant) donor antigens. In total, we propose that priming of host lymphoid populations to donor MiHA leads to the expansion of TCR^+CD8^+ effector T-cells which can exert potent barrier activity against MHC-matched marrow allograft in the absence of well defined, classic cytotoxic pathways.

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HIGH-DOSE BCNU, ETOPOSIDE, ARA-C, CYCLOPHOSPHAMIDE (BEAC) IS FEASIBLE IN AN OUTPATIENT COMMUNITY SETTING FOR PATIENTS (PTS) WITH RELAPSED AND HIGH-RISK NON-HODGKIN'S LYMPHOMA (NHL)

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Most studies of high-dose chemotherapy (HDC) and autologous stem cell transplant (ASCT) for NHL have been reported from university hospital inpatient settings. Between 1996 and 1999, US Oncology's network of community transplant centers (17 centers in 13 states) treated 103 NHL pts (median age 54 (range 23 - 70), sex: males-64, females-39) using an identical protocol (AOR-NHL-9605). Status of disease at time of transplant: 70 relapsed, 23 failed to achieve a CR with first line therapy, and 10 were in first CR. Pathology: 15 low grade, 68 intermediate grade, 3 high grade, 12 mantle cell, 4 T-Cell, and 1 unclassified lymphoma. All transplants were initiated in the outpatient setting with hospitalization only for complications. HDC consisted of bolus BEAC given as Day -7: BCNU 300 mg/m^2; and Day -6 to -3 : etoposide 100 mg/m^2 bid, ara-C 100 mg/m^2 bid, and cyclophosphamide 35 mg/kg qd. Median days to ANC > 500/μL was 10 (range: 7-29) and to platelets > 20,000/μL was 16 (range: 6-29). 59 pts never required hospitalization. Median days for 44 hospitalized pts was 10 (2-28). Four treatment-related deaths occurred prior to 100 days post-transplant. Median follow-up for 69 alive pts is 22 months (range 3-43).

High dose BEAC is a safe and effective regimen and that can be used in a community and outpatient setting.

	n	2 yr EFS	2 yr OS
All pts	103	48%	69%
Relapsed only	70	43%	62%
No 1st CR	23	44%	59%
1st CR	10	80%	90%
Mantle Cell	12	44%	100%
<60 y/o	77	54%	74%
60 or >	26	29%	55%

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PREDICTING RELAPSE AFTER ALLOGENEIC TRANSPLANT FOR ACUTE MYELOGENOUS LEUKEMIA

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Background: Allogeneic BMT is potentially curative in patients with AML.However,nearly 20-30% relapse within a year. There are no reliable markers to predict relapse. Lymphocyte recovery in early post-transplant period, development of acute GVHD (aGVHD), cytogenetics pre-transplant and disease status (advanced vs. non-advanced) have been shown to influence risk of relapse. Hypothesis: A scoring system using these variables can predict with more certainty the risk of relapse. Methods:A retrospective review of patients with AML who underwent matched related allogeneic-BMT at our institution between 1982 and 1999 was done to examine this hypothesis. Patients surviving up to 30 days post-transplant and those for whom all the variables were available were included for analysis. One point was assigned for each of the four good prognostic variable namely; normal cytogenetics pre-transplant or good-risk abnormalities (t15:17,t8:21,inv 16), non-advanced disease (AML in first remission), development of any grade of aGVHD by day +30 and absolute lymphocyte count (ALC) >175/μL at day 30 post-transplant. Results: Twenty-one relapses occurred among the 54 eligible transplants (Table). There was a continuous increase in the risk of relapse with decreasing total score. The relapse free survival as estimated by the Kaplan Meier product limit method demonstrated significant difference in the relapse survival between the groups (p <0.001, log rank test). Conclusion: A scoring system utilizing these four factors appears to be able to stratify patients according to increasing risk of relapse. However, these results needs confirmation using larger patient groups.

Score	Number of Patients	Relapse (%)	RR (95% CI)	P
0	2	2 (100)	69.5 (5.2, 925)	0.001
1	9	5 (56)	7.9 (0.92, 68.7)	0.06
2	12	6 (50)	6.4 (0.77, 53.2)	0.09
3	23	7 (30)	2.5 (.31, 20.4)	0.39
4	8	1 (13)	1	N/A

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CHARACTERISTICS OF CORD BLOOD COLLECTIONS: THE COBLT STUDY CORD BLOOD BANK EXPERIENCE

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In October 1997, recruitment for cord blood donors was initiated by the cord blood banks (CBBs) established as part of the NHLBI-sponsored Cord Blood Transplantation (COBLT) Study. After three years of recruitment at hospitals serving ethnically diverse populations, 30709 donors have been screened for donor eligibility. Of those screened, 18432 (60.0%) consented to donate cord blood, 9074 (29.6%) were not approached, and 3203 (10.4%) were approached but refused participation. As of November 1, 2000, 14609 cord blood units (CBUs) (38.9% White, 16.4% Black, 11.2% Asian, 20.3% Hispanic, 13.1% Mixed/Other/Unknown) have been collected from consented donors. The mean CBU collection volume is 64.2 mL (SD ± 31.2). CBUs with a collection volume ≥ 40 mL and ≥ 6 x 10^8 nucleated cells, or collection volumes ≥ 60 mL are red cell depleted and volume reduced. Of the collected CBUs, 9542 (65%) have been processed. The mean pre- and post-processing total nucleated cell count is 11.7 x 10^8 (SD ± 5.3) and 9.1 x 10^8 (SD ± 4.1), respectively. The mean viable nucleated cell recovery is 78.0% (SD ± 9.8). Flow cytometry characterization and colony forming unit enumeration are also available for 82% of the processed CBUs (CD 34+ cells: mean=2.6 x 10^6, SD ± 3.0; CFU-GM: mean=9.6 x 10^5, SD ± 8.4). Overall, 6841 (46.8%) CBUs have been discarded. A total of 4853 (70.9%) have been discarded prior to initiating processing, primarily for insufficient volume (n=4290, 88.4%). The remaining 1988 (29.1%) CBUs have been discarded post-processing primarily for a positive maternal infectious disease test (n=543, 27.3%). Of note, an additional 496 CBUs, currently under review, have a positive Hepatitis B core antibody test. Sixty-five percent of these CBUs are from Asian donors. Detailed data regarding donor recruitment, delivery, collection, processing and cryopreservation will be described.

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ROLE OF COSTIMULATORY AND CLASS I MHC MOLECULES IN THE INDUCTION OF TUMOR IMMUNITY TO MURINE ACUTE MYELOGENOUS LEUKEMIA

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Donor leukocyte infusions (DLI) have been used successfully to treat CML relapse after BMT, but have been less effective against AML. The inability of AML cells to induce leukemia-specific immunity may be due in part to the lack of necessary costimulatory molecules. In this study, we examined the effects of B7-1 and B7-2 costimulatory molecules on induction of immunity to C1498, a weakly immunogenic murine AML of C57BL/6 origin, with the intent of identifying a vaccine that would enhance

leukemia-specific immunity in donor/host-tolerant BM chimeras. We found that subcutaneous inoculation of syngeneic B6 mice with 10^6 viable C1498/B7-2 induced a strong CD8+ T-cell mediated immune response and persistent immunity to parental C1498 AML. CD4+ T-cells did not appear to play a significant role. C1498/B7-2 leukemia cells were sufficient for inducing immunity to C1498 since BM chimeras deficient in MHC class I+ APCs (ß2m ko) were able to reject viable C1498/B7-2 AML cells and a subsequent challenge with C1498. In contrast, inoculation of viable C1498/B7-1 resulted in progressive and lethal tumor growth. T-cells from mice inoculated with C1498/B7-1 leukemia were nonresponsive to stimulation with C1498/B7-2 or C1498 in vitro. When mixtures of B7-1+ and B7-2+ AML cells were coinjected subcutaneously, B7-2+ cells were rejected, while B7-1+ cells grew progressively. Using BM chimeras in which host T-cells lacked CTLA-4, we found that the failure of C1498/B7-1 cells to induce CD8+ T-cell immunity to C1498 was due to the interaction of CTLA-4 on the responding T-cells with B7-1 on the leukemia. In this model, specific interaction of B7-1 and CTLA-4 appears to result in a dominant negative effect and T-cell anergy. A differential effect of costimulatory molecules on induction of tumor immunity in vivo has significant implications for the design of vaccine strategies both within and outside the context of BMT/DLI therapy.

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QUANTIFICATION OF POLYOMA BK VIRURIA TO PREDICT THE OCCURRENCE OF HEMORRHAGIC CYSTITIS IN BONE MARROW TRANSPLANTATION PATIENTS

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Background. Polyoma BK virus is frequently identified in the urine of bone marrow transplantation (BMT) patients with hemorrhagic cystitis (HC). However, viruria is common even in asymptomatic patients, so that a direct causative role of BKV is difficult to establish. We tested the hypothesis that although qualitative testing of BK viruria is non-discriminating, quantification of BK viruria might be useful in predicting HC in BMT patients.

Methods. Prospective serial quantification of BK viruria in 24-hour urine and viremia was performed in fifty patients at a median of seven time points during BMT. A total of more than 800 patients samples were quantified for BKV VP-1 gene sequence with a real-time quantitative Polymerase chain reaction (Q-PCR).

Results. Twenty patients (40%) developed HC, of whom six had overt cystitis with gross hematuria (HC≥grade 2), and 14 had microscopic hematuria (HC grade 1). Patients with HC, when compared with asymptomatic patients, had significantly higher peak BK viruria ($6x10^{12}$ vs. 5.7×10^7 genome copies/day, $p < 0.001$) and larger total amount of BKV excreted during BMT ($4.9x10^{13}$ vs. $7.7x10^8$genome copies, $p < 0.001$). HC of severity ≥ grade 2 occurred after engraftment, and was significantly later than HC of grade 1 that usually occurred before engraftment (day 37 vs. day 4, $p < 0.01$). Multivariate analysis showed that HC was not related to age, conditioning regimens (cyclophosphamide and total body irradiation), type of BMT (allogeneic / autologous) and graft versus host disease. BK viruria, both peak value and total BKV excretion, was the only risk factor that predicted the occurrence of HC.

Conclusion. Quantification of BKV excreted in the urine of BMT patients predicted the occurrence of HC.

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GM-CSF AS PREEMPTIVE THERAPY FOR POST-TRANSPLANT EBV DISEASE

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Successful treatment of post-transplant EBV disease requires the control of B-cell proliferation and enhancement of EBV specific cytotoxic T-cell (EBV-CTL) immunity. Since GM-CSF can augment primary CTL responses and enhance CTL responses as an adjuvant to vaccines against viruses and cancer, we hypothesized that pre-emptive therapy with GM-CSF would enhance EBV-CTL immunity and decrease the incidence of EBV associated post-transplant lymphoproliferative disease (PTLD). Five pediatric patients, all T-cell depleted, matched unrelated donor marrow recipients, received GM-CSF ($250\mu g/m^2$ IV 3 times/week) at a median of 54 days post-transplant (range of 48-98 days) when they became EBV PCR positive and developed symptoms, i.e. fever, fatigue, nausea/vomiting, with no other identifiable etiology. Median EBV DNA levels by semi-quantitative PCR at time of symptoms were 350 copies/µg DNA (10-12200 copies). Median time following GM-CSF to resolution of all symptoms was 15 days (7-20 days) and EBV PCR negativity was 20 days (7-32 days). Four patients remained free of recurrence of symptoms or PTLD. One patient, after GM-CSF was discontinued, developed grade IV GVHD requiring increased immunosuppression, including ATG, and subsequently developed PTLD. One patient (Table below) had EBV-CTL quantitated using flow cytometry to detect $CD69^+$, $CD8^+$, and intracellular γ-IFN^+ cells following stimulation with an EBV transformed donor B-cell lymphoblastoid cell line (normal controls = 1-2% EBV-CTL). This patient developed symptoms on day +96 and started GM-CSF on day +98. In conclusion, these preliminary results suggest that GM-CSF may enhance EBV-CTL immunity post-BMT and may be an effective pre-emptive therapy for post-BMT EBV disease.

days post–BMT	EBV copies	%EBV–CTL	symptoms
+50	0	0	–
+97	12200	0	+
+117	0	8	–
+180	0	10	–

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MEASUREMENT OF AUTOLOGOUS IMMUNITY TO PRESENTATION LEUKAEMIC BLASTS IN PATIENTS IN FIRST COMPLETE REMISSION CAN PREDICT THOSE WHO WOULD BENEFIT FROM IMMEDIATE ALLOGENEIC STEM CELL TRANSPLANTATION

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Graft-versus-leukaemia after allogeneic BMT is known to be mediated by immune mechanisms. We have shown that immune-mediated anti-leukaemia activity is the mechanism of cure after autologous BMT. To further explore this phenomenon, we have studied 19 patients after chemotherapy alone. All patients were undergoing treatment for acute leukaemia (AML - 10; ALL -9) and the group consisted of 11 adults and 8 children (all cALL). Lymphocytes from each patient were collected in remission at completion of therapy and tested for in vitro cytolytic activity against their own presentation leukaemic blasts. Patients who subsequently relapsed were found to have significantly lower leukaemia-cytolytic activity (LCA) than those who remain in remission beyond two years ($p<0.001$). The subset of lymphocytes mediating this activity in vitro were CD56+/CD8wk+/CD3- natural killer cells (NK) and the cytolytic signal was mediated through CD8-alpha and CD69 molecules. The absence of LCA activity was able to predict relapse with a sensitivity of 100% and specificity of 91%. 7/11 (63.6%) of patients with LCA <12.2% relapsed within 2 years of attaining CR. The median time to relapse in this group was 11 months. Patients with LCA >12.2% all (13/13) remain in CR with median follow-up of 38 months (range 13-86). Patients who achieve remission after chemotherapy but fail to develop protective levels of NK-mediated LCA within 3-6 months of completion of chemotherapy should be considered as candidates for allogeneic transplantation or immunotherapy.

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RECIPIENT PRIMING AGAINST DONOR MHC ANTIGENS EFFECTIVELY AUGMENTS RESISTANCE TO ALLOGENEIC PROGENITOR CELL ENGRAFTMENT IN THE ABSENCE OF PERFORIN, FASL AND TNFR1 SIGNALING.

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Resistance against donor progenitor cell engraftment occurs following TCD of donor inoculum for GVH prophylaxis and in patients unintentionally primed against donor antigens. We are investigating radioresistant effector pathways in the barrier response against MHC mismatched allografts. B6-wild type recipients can resist up to 5 -10 x10^6 BALB/c TCD marrow allografts as evidenced by the absence of donor progenitor cells in recipient spleens 5 days post-BMT. Cytotoxically double deficient (cdd) B6 mice lacking perforin and FasL dependent function also demonstrated resistance, however, it was more easily overridden in these recipients as evidenced by increased CFU numbers following transplant of >5x10^6 donor marrow. Notably, the resistance in B6-cdd as well as B6-wt recipients against TNFR1$^{-/-}$ deficient donor progenitors was not different vs progenitors from TNFR1$^{+/+}$ normal donors. Therefore, a resistance pathway independent of perforin, FasL and TNFR1 signaling is present in recipients against MHC mismatched marrow allografts. B6-cdd, as well as B6-wt recipients, were then primed against donor antigens prior to BMT. Both groups of recipients exhibited markedly increased resistance as assessed by their rejection of greater than 3x10^7 allogeneic BM. B6-cdd primed recipients rejected TNFR1$^{-/-}$ donor marrow equally efficiently compared to TNFR1$^{+/+}$ marrow. We conclude that priming of recipients efficiently augments the non-perforin, FasL, TNFR1 pathway of resistance present in naive recipients. In total, these results support the notion that more than one effector pathway can contribute to resistance post- progenitor cell transplant. Resistance in the absence of three cytotoxic pathways is consistent with the notion that non-lytic mechanisms of inhibition (?reversible) may interfere with allogeneic progenitor cell engraftment

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T CELLS LACKING FASL AND PERFORIN ARE CAPABLE OF INITIATING SEVERE GVHD IN A MINOR HISTOCOMPATIBILITY MISMATCHED BMT MODEL.

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The mechanisms T cells employ to initiate and effect GVHD responses remain to be precisely defined. In vitro donor anti-host cytotoxicity assays do not always correlate with in vivo severity of GVHD, however cytotoxic molecules have been considered potential candidates for promoting host tissue damage following allogeneic BMT. We have examined the relevance of two effector mechanisms by utilizing cytotoxically double deficient (*cdd*) T cells in a minor antigen-mismatched BMT model system. Donor T cells from B6 mice expressing mutated fas ligand (B6-*FasL^{gld}*) and lacking perforin (B6-*pfp$^{-/-}$*) were compared to wild-type (B6-*wt*) T cells following injection into MHC-matched (H-2^b) C3H.SW hosts. Recipient mice received 9.0 Gy TBI on day –1 and either 5 x 10^6 B6-*wt* BM only, or B6-*wt* BM and 1.5 x 10^7 B6-*wt* T cells, or B6-*wt* BM and 1.5 x 10^7 B6-*cdd* T cells on day 0. All control BMT recipients survived (100%) however greater lethality was observed in recipients of B6-*cdd* (80%) compared to B6-*wt* (20%) T cells. Histopathological findings revealed more extensive mononuclear infiltrates and moderate to severe cellular atrophy in the GVHD target tissues examined (liver, stomach) in recipients of B6-*cdd* T cells vs. mild infiltrate and atrophy in recipients of B6-*wt* T cells. These data suggest that T cells lacking FasL and perforin function are capable of initiating GVHD to an equal or greater degree than B6-*wt* T cells in a minor antigen-mismatched BMT model. Interestingly, although BMT recipients of both B6-*wt* and B6-*cdd* T cells contained elevated ratios of CD8:CD4 T cells, the latter recipients exhibited increased donor cell proliferation and increased host cell survival. These two factors may have contributed to the large increase in spleen cell numbers and the increased severity of GVHD following BMT containing B6-*cdd* T cells.

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ELEVATED PLASMA IL-6 CONCENTRATIONS ARE AN EARLY MARKER OF ORGAN DYSFUNCTION AFTER ALLOGENEIC STEM CELL TRANSPLANTATION.

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Introduction: Organ dysfunction (OD) after allogeneic transplantation is associated with morbidity and death. Clinically, OD is preceded by weight gain and an increased respiratory rate which may be cytokine related. Consequently, we measured plasma cytokine concentrations at the time of admission, at the end of preparation, 5 days before OD, on the day of OD, and 5 days after the onset of OD and compared them to controls who never developed OD.

Methods: Plasma was collected daily in patients undergoing stem cell transplantation. Patients were evaluated daily for pulmonary dysfunction (O2 saturation <90), CNS dysfunction (drop of 4 points on Folstein MMSE) and hepatic dysfunction (VOD by the Jones Criteria). Plasma concentrations of TNF-alpha, IL-6, and IL-10 were measured using an ELISA (R&D Systems, Minn MN). The lower limit of sensitivity was 5pcg/ml or less for all assays and intra-assay precision between 5-10%. Cytokine concentrations were compared between OD and control patients using the Wilcoxon Rank Sum and Fisher's Exact Test.

Results: The number of patients with measurable IL-6 levels prior to preparation and median values 5 days prior to the onset of OD were significantly greater in the OD group (p=0.022 and p=0.0033, respectively). This significant elevation continued at the time of OD (p=0.0036) and 5 days later (p=0.0002). There were few measurable TNF-alpha and IL-10 levels prior to preparation or 5 days prior to onset of OD. There were significantly more patients with detectable IL-10 and TNF-alpha levels at the time of OD and 5 days later compared to controls.

Conclusions: IL-6 is an earlier predictor of OD than either TNF-alpha or IL-10. IL-6 levels were significantly elevated prior to transplant and 5 days prior to onset of OD which may allow the development of aggressive preventive strategies in these high risk patients.

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EX VIVO T-CELL DEPLETION FOR GRAFT VERSUS HOST DISEASE PROPHYLAXIS IN RELATED HAPLO-IDENTICAL ALLOGENEIC STEM CELL TRANSPLANT RECIPIENTS.

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Haploidentical stem cell transplantation is complicated by graft rejection, GVHD, relapse, and prolonged immunodeficiency. Aversa et al (NEJM 1998; 339: 1186-93.) report high stem cell doses and T-cell depletion via CD34+ cell selection reduce graft rejection and GVHD. Since Dec 1999, we performed haploidentical transplants in 9 patients (pts), median age=24yo, with high-risk hematologic malignancies (AML=4, NHL=3, ALL=2). Pts received TBI 800cGy with lung shielding to 400cGy (n=7) or melphalan 140mg/m^2 /d (n=2) (day –7), thiotepa 10mg/kg/d (day –6), fludarabine 40mg/m^2 /d (day –5 to –1), and rabbit ATG (ThymoglobulinTM) 2.5 mg/kg/d (day –5 to –2). Eight pts received tacrolimus 0.03mg/kg/d from day –7 to –1. No GVHD prophylaxis or growth factor support was adminstered. Cell dose targets were 10x10^6 CD34+ cells/kg and a maximum of 5x10^4 CD3+ cells/kg following T cell depletion via CD34+ cell selection (CliniMACS, Milytenyi). Patients received a mean of 10.7x10^6 CD34+ cells/kg (median CD34+ cell recovery =55%) and 3.3x10^3 CD3+ cells/kg (median 5 log reduction). One pt died prior to engraftment. Of 8 evaluable pts, the median time to ANC>500 was d12

post PBSCT. Five of 8 evaluable pts achieved plt>20K by a median of d21 post PBSCT. Three pts had delayed platelet recovery. No pt has developed GVHD. Significant infectious toxicity occurring by d100 included aspergillosis = 5 pts, CMV pneumonia = 3 pts, non-CMV viral pneumonia = 2 pts, bacterial infections = 2 pts, candidemia = 1 pt, and acyclovir-resistant HSV in 1 pt. Two of nine pts are alive 101d and 197d post PBSCT. Seven pts have expired due to relapse (1), multi-organ failure (1), PCP (1), IP/HUS (1), IP (1), CMV pneumonia (1), or EBV lymphoma (1). With this approach, pts experienced prompt neutrophil engraftment and experienced no GVHD. However, delayed immune reconstitution and infectious complications remain formidable challenges.

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OSTEOPOROSIS IN THE STEM CELL TRANSPLANT PATIENT: A PROTOCOL FOR EVALUATION, PREVENTION AND MANAGEMENT

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Osteoporosis is a skeletal condition characterized by decreased density of normally mineralized bone. The reduced bone density leads to decreased mechanical strength, thus making the skeleton more likely to fracture. Osteoporosis can be a significant clinical problem in the long-term follow-up of both men and women who have undergone a stem cell transplant. It is associated with significant medical costs, and can have a detrimental effect on long-term quality of life. Patients undergoing allogeneic transplantation have multiple risk factors for osteoporosis, including systemic corticosteroid therapy for the treatment of graft versus host disease. Significant advances have been made in our ability to detect, prevent and treat osteoporosis. However, to be most effective, therapy should be started early, before serious bone loss has occurred. Since there can be a latent period before the appearance of pathologic fracture, and since many different post-transplant issues compete for our attention in these complex patients, insufficient attention may be given to osteoporosis prevention and treatment until symptoms occur. This paper provides an overview of the pathophysiology of osteoporosis, and presents an algorithm for its evaluation, prevention and management in the stem cell transplant patient. The algorithm incorporates (1) risk assessment and screening, (2) diagnostic strategies, (3) preventive measures such as exercise and fracture prevention counseling, and (4) the use of pharmacologic agents, including hormone replacement, calcium supplementation, bisphosphonates, calcitonin, vitamin D analogs, and selective estrogen receptor modulators. The importance of patient education, endocrinology consultation, and follow-up is emphasized. With greater knowledge of the factors that affect bone health and the options for screening and therapy in patients at risk for or experiencing osteoporosis, the severity and consequences of this problem can be minimized.

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ADHERENCE ISSUES IN STEM CELL TRANSPLANTATION: THEORETICAL PERSPECTIVES AND CLINICAL INTERVENTIONS

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Stem cell transplantation (SCT) provides long-term disease-free survival for individuals with a variety of hematologic and non-hematologic conditions. However, despite advances in technology and clinical care over the past twenty years, the psychological stresses of transplant remain. The procedure of stem cell transplantation entails a prolonged, aversive treatment process; a long and frequently complicated physical and psychological recovery post-discharge; prolonged reliance on the healthcare system; and unpredictability of the transplant course and eventual outcome. Clinical experience, as well as the literature suggests that this is a situation in which adherence problems often develop. The daily struggles that can ensue between patient and staff are a major focus for the caregiving team and may have life-threatening implications.

This paper presents an overview of the adherence issues involved in SCT. After a review of the demands for adherence imposed by the SCT regimen, the cognitive, socio-behavioral, environmental and illness-related factors that may influence adherence in the SCT patient population are discussed. The paper then summarizes the five major theoretical orientations within which the problem of non-adherence can be conceptualized. The paper concludes by describing an interaction model designed to facilitate a therapeutic alliance, as well as presenting some partnership and problem-solving strategies that health care providers can use to create a supportive climate conducive to adherence, as well as perceived control. Case summaries are used to highlight significant clinical concepts, relevant ethical issues, and multi-disciplinary intervention strategies.

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REAL-TIME PCR-GUIDED PREEMPTIVE THERAPY FOR CYTOMEGALOVIRUS FOLLOWING ALLOGENEIC STEM CELL TRANSPLANT

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We previously reported that cytomegalovirus (CMV) antigenemia infrequently precedes the onset of CMV gastroenteritis, and that real-time PCR detects viral reactivation earlier than antigenemia. We report here the results of prospective preemptive therapy (PT) for CMV diseases based upon real-time PCR. Twenty-nine patients who underwent allogeneic stem cell transplantation (related; 11, unrelated; 18) were evaluable. After engraftment, real-time PCR using DNA extracted from plasma was monitored weekly, and once it revealed positive, monitored twice a week. The sensitivity of PCR method used in this assay was 200 copies/ml. CMV antigenemia was simultaneously monitored. Only patients who developed grade II-IV acute GvHD were considered at high-risk of developing CMV diseases, and were designed to receive PT based upon real-time PCR. If real-time PCR revealed positive, ganciclovir (GCV) was initiated at a dose of 5mg/kg/day for 14 days. If viral copies continued to increase after initiation of GCV, the dose of GCV was increased up to 10mg/kg/day. Of the 29 patients, 22 developed grade II-IV acute GvHD (group A), and 7 developed grade O-I (group B). In group A, 19 (83%) developed positive PCR, and received PT. One patient in group A developed CMV disease (gastroenteritis), in whom real-time PCR did not precede the onset of gastroenteritis. Except in 2 cases, antigenemia did not precede the real-time PCR, and it did not correlate with viral copies assessed by real-time PCR after initiation of GCV. In group B, only 1 patient developed positive PCR without developing positive antigenemia, which became negative without treatment. In conclusion, these results suggest that real-time PCR-guided PT was effective in the prevention of CMV diseases including gastroenteritis. However, it should be applied only to high-risk patients to reduce the overtreatment.

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INDUCTION CHEMOTHERAPY FOLLOWED BY MINI-ALLOGENEIC STEM CELL TRANSPLANTATION (ALLO-SCT) - A SUCCESSFUL TREATMENT STRATEGY FOR OLDER AND HIGH RISK ADULTS WITH DIAGNOSIS (DX) OF MYELOID MALIGNANCIES

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Allo-SCT is a curative option for patients with poor prognostic myeloid malignancies. However, older patients and patients with comorbid conditions are not offered potentially curative treatment options.

We report on four high-risk patients. Two were older than 65 years: UPN1 (70 yrs; dx secondary AML), UPN2 (66 yrs; dx blast-phase CMML). Two had prior solid organ transplantation and comorbid conditions: UPN3 (kidney transplant, h/o lupus, stroke,

DLCO of 35%; dx MDS) and UPN4 (liver transplant, h/o hepatitis C; dx AML with 11q23 abnormality). All received remission-inducing chemotherapy Ara-C/mitoxantrone (UPN1, UPN2), fludarabine/Ara-C/mitoxantrone with allo-SCT (UPN3), and mitoxantrone/etoposide/topotecan (UPN4). Mini-allo SCT was performed using Fludarabine 25mg/m^2 for 5 days, Melphalan (140-180mg/m^2) and peritransplant horse ATG (30-60mg/kg). Patients were infused a median of 8.42 (range 6.52 to 12.88) x 10^6 CD34 cells/kg. Graft versus host disease (GVHD) prophylaxis consisted of mycophenolate mofetil from day -1 to 30 and cyclosporine. 3/4 patients were in CR and one patient (UPN3) in cytogenetic relapse (after an initial CR) at the time of transplant. Neutrophil engraftment (>1,000/μL) occurred at a median of 10 days (range 9 to 11) and platelet engraftment (>50,000/μL) at a median of 20 days (range 16 to 21). 2/3 evaluable patients developed overall grade II GVHD which responded to steroids. Two patients developed chronic GVHD (UPN1, limited; UPN3, extensive). UPN1 had a complete response to therapy with steroids. UPN3 was taken off immunosuppression on day 92 and developed chronic GVHD around day 130, which responded to cyclosporine and prednisone. Three patients with follow-up >100 days (UPN3, 283; UPN1, 282; UPN2, 144) are alive and disease-free with 100% donor chimerism documented on days 170, 169 and 134, respectively. Conclusion: Induction chemotherapy followed by mini-allo SCT can be performed safely and may be curative in older and high risk patients with myeloid malignancies.

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PERIPHERAL CD 34 CELL CONCENTRATIONS CORRELATE CONSISTENTLY WITH CD34 YIELD IN APHERESIS PRODUCT INDEPENDENT OF MOBILIZING REGIMEN

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Peripheral blood (PB) CD34 concentrations were measured by flow cytometry and compared with CD34 yield in the apheresis product (AP) in 156 stem cell collection procedures. Twenty-nine of these were done on allogeneic sibling donors who received 10 mg/kg G-CSF for 5 days as mobilizing regimen (Allo G). Of the 127 autologous stem cell collections, 60 were mobilized with the same dose of G-CSF as the sibling donors (Auto G), 29 received cytoxan 2 gms/m^2 + Taxol 175 mgs/m^2 followed by 10 mg/kg G-CSF (CTG), and 38 were mobilized with cytoxan 2 gms/m^2 + 10 mg/kg of G-CSF (CG). Target cell dose was 5 x 10^6 CD34 cells for allos and 2 x 10^6 CD34 for autologous donors. Number of aphereses required to achieve target dose were compared between groups. Results are shown in the table. PB concentration of CD34 was found to have linear correlation with CD34 concentration in the AP in all 4 groups. Coefficient of correlation (r^2) was equal to or greater than 0.90. Auto G yielded the lowest CD34 cells and required the most apheresis to achieve target dose. CD34 yield from allogeneic donors was significantly higher than Auto G donors. The highest CD34 yield was in CG group. Our data shows that the best predictor of CD34 yield in AP is the PB CD34 concentration. PB CD34 concentration can be used to time the stem cell collections in donors mobilized with chemotherapy and G-CSF.

	CG	CTG	AUTO G	ALLO G
n	38	29	60	29
PB CD34 (med, 10^6/kg	.05	.03	.01	.08
AP CD34 (med, 10^6/kg	554	257	33.65	259
r^2	0.90	0.92	0.96	0.90
# of apheresis	1	1	3	2

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SUCCESSFUL ENGRAFTMENT AND FEW DONOR COMPLICATIONS ASSOCIATED WITH AUTOLOGOUS AND ALLOGENEIC STEM CELL TRANSPLANTS USING VERY YOUNG DONORS

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Very young children are being utilized as peripheral blood stem cell (PBSC) donors for autologous or allogeneic transplantation. We report on 15 patients, ages 7 months to 8 1/2 years, who have undergone successful leukapharesis. They ranged in size from 6.4 - 22kg. Thirteen of 15 patients had the PBSC's infused after ablative therapy and all were successfully engrafted. Six patients were mobilized using their chemotherapy protocols {VCR/CYT/CPDD/VP (2); CPDD/VP (3); VCR/CYT/ADR(1) and 6 received priming with CYT alone. The 3 normal donors were mobilized with GCSF. Thirteen of 15 children required placement of an apheresis catheter. There were no complications associated with placement or use of these catheters. The large volume apheresis procedures were associated with minimal complications such as hypocalcemia (2), agitation requiring conscious sedation (2), mild dehydration (1). Three children underwent collection on a single day, 9 had two collections and 3 had three days of collection. No long term complications have been noted. Cell doses ranged from 1.4 - 49 x 10^6 CD34+ cells/kg of recipient weight. In our experience, PBSC collection can be performed safely on very small children and with only minor complications.

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SIGNIFICANT IMPROVEMENT IN PULMONARY FUNCTION AFTER ACITRETIN THERAPY IN A CHILD WITH BRONCHIOLITIS OBLITERANS SYNDROME (BOS)

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We report a 14 year old boy who developed severe mixed obstructive/restrictive pulmonary defect 14 months after 6/6 MRD sibling transplant for ALL in second remission. He presented with wheezing, cough, low grade fevers, pneumomediastinal and apical pneumothoraces. FEV_1 decreased from 118% (pre-BMT) to 56% at the time of presentation. Bronchoalveolar lavage revealed no evidence of infection and a high resolution CT showed a mosaic pattern consistent with BOS. The patient's immunosuppressive regimen was broaded to include ATG (4 day course), methylprednisolone and tacrolimus. His Lansky score declined from 100% (12 months post-BMT) to 50% with marked activity intolerance and he developed a requirement for supplemental oxygen. Acitretin was started at 0.6mg/kg/day; prednisone and tacrolimus were continued. Inhaled corticosteroid and β agonist therapy was also initiated. FEV_1 and FVC nadirs of 0.51L and 1.01L improved to 0.76L and 1.82L within 6 months. Measures of restrictive pulmonary disease also improved over the 6 month period: SVC (46%→77%); IC (47%→75%); ERV (45%→83%); RV (280%→179%); RV/TLC ratio (63%→40%). The TLC remained stable at 2.9L. During this same period, his performance scale increased to 70% and oxygen was discontinued. The clinical course of BOS post-BMT ranges from slow progression to diffuse necrotizing fatal bronchiolitis. We hypothesize that the addition of acitretin, a retinoid, who known functions include normalization of epidermal differentiation and decreased epidermal inflammation, contributed to the improvement in this patient's pulmonary function. Acitretin may be of benefit in patients experiencing severe decrement in pulmonary function in post-BMT BOS.

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GRANISETRON PLUS DEXAMETHASONE VS. GRANISETRON ALONE IN THE PREVENTION OF VOMITING INDUCED BY CONDITIONING FOR STEM CELL TRANSPLANTATION: A PROSPECTIVE RANDOMIZED STUDY

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Nausea and vomiting are acute symptomatic problems for patients receiving high-dose chemotherapy or chemoradiotherapy prior to stem cell transplantation. We have previously demonstrated that granisetron is superior to standard antiemetics consisting mainly of methoclopramide against the vomiting induced by conditioning for stem cell transplantation. This prospective randomized study compared the efficacy and toxicity of granisetron and dexamethasone with granisetron alone for antiemetic control in patient receiving high-dose chemotherapy with or without total body irradiation for stem cell transplantation. Patients were randomized to receive granisetron at a dose of 40mg/kg with or without dexamethasone (8 mg) orally (GS vs G

group) starting 30 min prior to each dose of chemotherapeutic agent or TBI. Fifty patients were evaluated for analysis. During the first 24 hours of conditioning, 23 of 25 patients (92%) in the GS group achieved a complete control of emesis (no emetic episodes a day) compared with 72% in the G group (p=0.06). For patients receiving TBI on the first day of conditioning, a complete emetic control was achieved in all patients (100%) in the GS group compared with 63% in the G group (p=0.02). The same degree of emetic control was maintained throughout conditioning period in 65% of the GS group compared with 45% of the G group (p=0.10). Adverse reactions were observed more frequently in the GS group (60% vs 5%), which included insomnia, headache, flushing, and hyperglycemia. None of the events were serious. We conclude that granisetron and dexamethasone seems superior to granisetron alone for the prevention of emesis resulting from conditioning, however, the side effects were more frequent which may limit the wide use of this combination.

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IMMUNOLOGICAL MECHANISM OF MS FLARES ASSOCIATED WITH G-CSF IN STEM CELL TRANSPLANT PATIENTS

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High dose immunosuppression with peripheral blood stem cell transplantation (SCT) has been given as investigational therapy to over 100 multiple sclerosis (MS) patients worldwide. In most of these patients, cyclophosphamide or Prednisone has been used along with granulocyte colony stimulating factor (G-CSF) for stem cell mobilization. Four of 10 patients who received G-CSF alone developed transient flares of MS disease activity (Neurology 2000; 54: 2147-2150). We present results here to suggest a possible mechanism.

Methods: Peripheral blood mononuclear cells (PBMC) were obtained before the first G-CSF dose and 4 days later before apheresis in 6 subjects (2 with MS and 4 normal controls). These paired samples were assayed for lymphoproliferative responses to myelin antigens and cytokine production by ELISPOT.

Results: Four days of G-CSF increased counts of total leukoctytes 5-fold, neutrophils 7-fold, monocytes 4-fold, and lymphocytes 1.5-fold. With G-CSF, there was suppression of PBMC proliferative reponses to epitopes spanning myelin basic protein as well as immunodominant peptides of proteolipid protein and myelin oligodendrocyte glycoprotein. ELISPOT assays consistently showed a marked increase in cytokine secreting cells after G-CSF, including the Th1 cytokines IL-2 and interferon-gamma and the Th2 cytokines IL-4 and IL-10.

Discussion: Increases in inhibitory monocytes may account for the suppression of PBMC proliferative responses during G-CSF administration. In contrast, PBMC cytokine production was upregulated after G-CSF, including Th1 cytokines that have been associated with MS exacerbations. Our hypothesis is that shifts in PBMC population brought about by G-CSF influence the monocyte-lymphocyte infiltrate of the MS plaque. After SCT, we have found lymphocytes to be absent and macrophage-monocytes sparse in MS plaques. However, at the time of stem cell mobilization, most patients eligible for protocol treatment would be expected to have active inflammatory plaques. Changes in the cytokine microenvironment of these plaques may explain the disease flares seen with G-CSF.

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REPIFERMIN (KERATINOCYTE GROWTH FACTOR-2) PROMOTES MUCOSAL HYPERPLASIA THROUGHOUT THE ALIMENTARY TRACT IN NORMAL MONKEYS

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Complications arising from chemotherapy or radiation-induced mucositis often limit cancer treatment. The development of a single agent that promotes mucosal healing following chemo- or radiation therapy is desirable. Repifermin is an FGF-like epithelial mitogen that has been shown to reestablish intestinal mucosa in a rodent ulcerative colitis model. The goal of this study was to determine whether daily administration of repifermin promotes epithelial hyperplasia within the alimentary tract of normal cynomolgus monkeys. Vehicle (IV only) or repifermin was administered intravenously (20, 75 or 200 µg/kg/d) or subcutaneously (750 µg/kg/d) once daily to normal cynomolgus monkeys over 29 days. A 28 day recovery subgroup was also included for the vehicle as well as SC and 200 µg/kg/d IV groups. Repifermin-induced changes were noted throughout the alimentary tract. Epithelial hyperplasia in the buccal mucosa and dorsal aspect of the tongue was noted in all animals receiving repifermin. Esophageal mucosal thickening was observed at necropsy in the high dose IV group. This correlated histologically with minimal to mild esophageal epithelial hyperplasia that was noted in all treatment groups (including SC). The incidence and degree of this hyperplastic response were dose-dependent in the IV-treated animals. No mucosal changes were seen in the stomach. Additionally, repifermin induced mucosal and goblet cell hyperplasia throughout the intestine. Following the recovery period, the incidence and degree of the hyperplastic changes were substantially reduced or completely resolved. These results indicate that repifermin stimulates reversible mucosal hyperplasia throughout most segments of the alimentary tract of normal monkeys. Thus, repifermin may be useful in treating chemo- and radiation therapy-induced mucositis.

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IMPLICATIONS OF GLOBAL PRICING IN PEDIATRIC STEM CELL TRANSPLANTATION: RISK MINIMIZATION STRATEGIES

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Introduction: Over the past 20 years, healthcare reimbursement has shifted from fee-for-service to prospective payment with increased risk sharing between payors and providers. Global pricing (GP) is a strategy in which a price (base and outlier rate) is negotiated for all contracted services within a designated time frame; the provider assumes the financial risk. We developed GP for pediatric stem cell transplant (SCT) services, emphasizing risk minimization.

Methods: Charge and cost data were compiled for 237 SCTs (209 patients) from FY 96-99 from the date of admission through day 100.The primary focus of pricing alternatives was the peri-SCT hospitalization since it contributed >90% of total costs. A Monte Carlo simulation of different pricing assumptions was undertaken. For each value selected, the model calculated a profit or loss: (Base rate + outlier rate)-total cost =profit or loss. Hypothetical simulations for unrelated donor SCT (N=66) demonstrate the impact of parameter changes on the probability of running a loss (PLOSS).

Results: Using the median length of stay (LOS) of 40 days and median charges of $160,000, a change in the outlier rate from $2500 to $3500 would decrease the PLOSS from 10% to 5%. If LOS and the outlier rate were held constant (40 days; $2500), the PLOSS could be maintained at 5% with a base rate increase from $160,000 to $200,000. An increase in the period covered by the base rate to 60 days (75th percentile) would necessitate an adjustment in base rate to $250,000 with a $2500 outlier or $225,000 with a $3500 outlier to minimize the PLOSS to 5%.

Conclusions: To successfully negotiate contracts for SCT under a GP mechanism, it is essential to understand the risks associated with certain negotiation requests. This model allows us to vary assumptions to develop competitive, but fiscally responsible prices.

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OUTPATIENT AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION IN A COMMUNITY SETTING

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In order to be cost-effective, high dose chemotherapy and autologous blood stem cell transplants (ASCT) are frequently being performed on an outpatient basis. We report our experience of outpatient ASCT in a private practice-community hospital setting. From September 1997 to September 2000, 42 individuals received high-dose chemotherapy with autologous blood stem cell transplant. Of these patients, 9 were diagnosed with relapsed non-Hodgkin's lymphoma (NHL), 2 with relapsed Hodgkin's disease (HD), 6 with multiple myeloma (MM), and 25 with breast cancer

(BC). The median age was 48 years (range 17-67 years) with 29 females and 13 males. The transplant conditioning regimens consisted of BEAM (BCNU, Etoposide, Ara-C, Melphalan) for NHL and HD; Etoposide and Melphalan for MM; TTCM (Taxol, Thiotepa, Carboplatin, Melphalan) for BC (8 patients)and Stamp V (Carboplatin, Cyclophosphamide, Thiotepa) for BC (17 patients). The median day to white blood cell engraftment (ANC>1000/μL) was 11 with a range of 7-21 days. The median day to platelet engraftment (PLT>20K/μL) was 22 with a range of 8-210 days. One patient died within 100 days of transplant due to sepsis and progressive disease. During the first 100 days post-transplant, 14 of the 41 patients treated as outpatients (34%) remained outpatients. Sixty-six percent, or 27 patients, required hospitalization with an average length of stay of 8 days. The most frequent admitting diagnoses were culture negative sepsis (48%), culture positive sepsis (18%), bleeding (15%), and mucositis (11%). Overall survival for this small number of patients with limited follow-up is as follows: NHL 67%, HD 50%, MM 100%, BC 88%. We conclude that high dose chemotherapy and autologous blood stem cell transplantation is feasible and appears safe to be performed as an outpatient in a community hospital setting.

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THE IMPACT OF HISTOLOGY ON HIGH-DOSE THERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH RELAPSED NON-HODGKIN'S LYMPHOMA: EXPERIENCE OF A SINGLE TRANSPLANT CENTER

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Purpose: To evaluate the relapse-free survival (RFS), overall survival (OS) and transplant-related mortality (TRM) after high-dose therapy and autologous stem cell transplant (SCT) in patients with Non-Hodgkin's lymphoma (NHL).

Methods: A total of 48 patients with relapsed NHL (28 with intermediate/high-grade lymphoma [I-HGL] and 20 with low-grade lymphoma [LGL], according to International Working Formulation) were treated between 1993 and 2000. The median age at transplant was 49 years (range 17-65). There were 23 females and 25 males. Fourty-two patients were in first relapse, chemosensitive or untreated, and 6 were in second or higher chemosensitive relapse. The median interval between initial diagnosis and transplant was 31 months (range 4-185). The conditioning regimens used were VP/Cy/TBI in 29 patients, TT/TBI in 8 patients and VP/Cy/TT in 11 patients. Five patients received unmanipulated stem cells from bone marrow (BM) only, 20 from peripheral blood (PB) only, 22 patients from both BM and PB, and one patient died prior to HSC infusion. The median duration of follow up was 20 months (range 1-92).

Results: The median time to engraftment (ANC >500) was 12 days. Using Kaplan-Meier analysis, for all patients who underwent high-dose therapy, the 5-year RFS was 45% +/-5%, and 5-year OS was 58% +/- 5%. The 5-year RFS for patients with I-HGL was 35% +/-4% and for LGL was 55% +/- 7%, P>0.05. The 5-year OS for patients with I-HGL was 42% +/-7%, and for LGL was 78% +/-7%, P<0.05. Five patients (10%) died of transplant-related causes. Using multivariate analysis, there was no impact of age, sex, stem cell source or conditioning regimen on RFS, OS or TRM.

Conclusions: HDT with ASCT is highly effective in patients with relapsed low or intermediate/high-grade NHL, with acceptable transplant-related toxicity. The RFS and OS were superior in patients with low-grade NHL.

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SUCCESSFUL REPRIORITIZATION OF ALTERNATE DONOR SELECTION TO UNRELATED CORD BLOOD FOR HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) IN CHILDREN AND ADOLESCENTS WITH LEUKEMIA

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Allogeneic HSCT can cure refractory leukemia. The optimal alternate HSC source for patients without a matched sibling (MS) is uncertain. Unrelated cord blood (UCB) is increasingly available and may have a lower GVHD risk with decreased toxicity. We explored reprioritizing UCB as the alternative donor HSC source over MUD or T cell depleted HLA disparate relatives (Fam). We report results of 61 consecutive HSCT for leukemia. Conditioning was with TBI, Cytarabine, Cyclophosphamide, ATG (6/26 MS Bu/Cy). UCB patients received 2.45 (0.7–12.5) $x10^7$ TNC*/kg, with 13/23 UCB ex vivo expanded. (*median range)

We conclude that in patients receiving consistent cytoreduction and supportive cares, UCB produces results similar to MS HSCT. Despite a wide range of TNC/kg, engraftment occurred with all UCB, although it may be delayed. The low relapse rate with UCB is encouraging, particularly with the reduced GVHD risk. We continue efforts to accelerate engraftment through ex vivo expansion and to minimize non-relapse mortality.

	UCB	MUD/Fam.	MS
N	23	12	25
Age*(yrs)	8(0.5–24)	12.5(2–23)	8(1–22)
Engraftment	21/21	9/10	23/23
ANC*(days)	25(14–49)	15(12–26)	23(13–36)
AGVHD ≥ 2	5/19(26%)	5/10(50%)	11/23(48%)
CGVHD	6/16(38%)	4/4(100%)	5/20(25%)
Non–relapse Mortality	4/23(17%)	10/12(83%)	5/23(22%)
Relapse	3/16(19%)	0/4	4/20(20%)
EFS (1 yr/2yr)	62.5/62.5%	22.5/18%	67/62%

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THE STEM CELL CRYOPROTECTANT DMSO (DIMETHYLSULFOXIDE) ACTIVATES MORPHOLOGIC GRANULOCYTIC DIFFERENTIATION THROUGH THE RETINOIC ACID RECEPTOR ALPHA

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DMSO is a low molecular weight organic compound, which is used widely as a cryopreservative for hemaotpoietic stem cells and other cells. It has antioxidant properties and is capable of inducing granulocytic differentiation, growth inhibition, and apoptosis of HL-60 myeloid leukemia cells. We have previously characterized a subclone of HL-60 cells, HL-60R, that is resistant to retinoic acid (RA)-induced myeloid differentiation. The basis of the resistance to RA involved a point mutation in the ligand-binding domain of RAR-α to create a dominant negative RAR-α (RAR-α411). To determine whether DMSO-induced myeloid differentiation might also involve RAR-α, we treated HL-60R cells with DMSO and found them to be relatively resistant to DMSO-induced morphologic differentiation. Moreover, the DMSO response could be rescued by transducing HL-60R cells with the retroviral vector LRARαSN. To determine whether this was a specific effect of DMSO with RAR-α, we transduced HL-60 cells with a retroviral construct containing the dominant negative RAR-α411 which conferred resistance to DMSO-induced morphologic granulocytic differentiation, but resulted in profound growth inhibition upon treatment with DMSO. To determine whether DMSO would activate transcription through RAR-α, we co-transfected RAR-α and an SV40 promoter driven luciferase gene containing an upstream RA response element. DMSO activated luciferase gene expression as measured by luminescence compared to untreated, ethanol treated, and RA-treated controls. We conclude that DMSO induced morphologic myeloid leukemia cell differentiation is mediated in part through RAR-α, which may have implications for use of DMSO as a cryopreservative.

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TOLERANCE INDUCTION BY HUMAN CD34+ CELLS AND ANTI-CD40L ANTIBODY PLUS CTLA-4 IG: PRECLINICAL STUDIES

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We previously demonstrated that human CD34+ cells induce allogeneic T cell proliferation in vitro. In this study we addressed

the hypothesis of inhibiting allogeneic T cell reactivity to CD34+ cells and inducing T cell tolerance by blocking CD40:CD40L and/or B7:CD28 pathways. In vitro primary mixed lymphocyte cultures (MLC)(n=4 exp.) were performed with irradiated normal G-CSF-mobilized-CD34+ blood cells, or mononuclear cells (MNC) from the same donor, and HLA mismatched allogeneic CD4+ T cells or MNC as responders. Anti-CD40L (CD154) monoclonal antibody (BIOGEN Inc., Cambridge, MA) induced on average 55±20% and 64±20% inhibition of T cell response to CD34+ cells and to MNC, respectively, but a higher dose of the antibody was required with CD34+ cells (50 μg/mL) than with MNC (2 μg/mL). T cell alloreactivity was similarly reduced also by CTLA-4 Ig (2 μg/ml)alone, whereas combination of CD154 and CTLA-4 Ig potently blocked T cells after stimulation with either CD34+ cells (86±9% inhibition) or with MNC (84±10%) in primary MLC. Then, responder cells were rechallenged with irradiated stimulators from the same donor in secondary MLC. Only responders obtained from cultures with CD34+ cells or MNC plus CD154 and CTLA-4 Ig were unresponsive(n=4 exp.), whereas comparable responses to third party stimulators were obtained by anergic or control T cells (n=2 exp.). Interestingly, addition of IL-2 (50 UI/mL) did not seem to reverse T cell unresponsiveness. Finally, we demonstrated that irradiated CD34+ cells induce potent allogeneic cytotoxic responses in a standard 51Cr release assay (n=4 exp). In these experiments, cytotoxicity could be prevented by the presence of CTLA-4 Ig alone. In conclusion, since CD154 and CTLA-4 Ig have a synergistic effect in inducing T cell anergy after stimulation with human CD34+ cells in vitro, new strategies for inducing tolerance across HLA barriers may be investigated in allogeneic hematopoietic CD34+ cell transplantation.

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PULMONARY INFILTRATES (PI) IN HEMATOPOIETIC CELL TRASPLANTATION (HCT) RECIPIENTS: CLINICAL IMPACT OF A PROSPECTIVE, SEQUENTIAL DIAGNOSTIC APPROACH.

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Over a 30-month period, we prospectively evaluated all consecutive HCT patients with PI with the same diagnostic approach that included non-invasive and bronchoscopic techniques. Non-invasive methods were: serologic tests; blood antigen detection; blood, sputum, nasopharyngeal wash (NPW) and tracheobronchial aspirates (TBAS) cultures. Bronchoscopic techniques included protected specimen brush (PSB) and bronchoalveolar lavage (BAL). Overall, 61 patients with PI were evaluated: 52 had received an allogeneic HCT and 9 an autologous HCT. An etiological diagnosis was obtained in 47 (77 %) patients. Etiology was infectious in 33 (70 %) and non-infectious in 14 (30%) . Fourteen episodes (23%) remained undiagnosed. Main infectious etiologies were viral (36%), fungal (24%), bacterial (21%), and polymicrobial (15%). Most frequent pathogen were Aspergillus fumigatus (n= 6), cytomegalovirus (n=6) and Candida spp (n=3). Most common non-infectious etiologies were pulmonary edema (23%) and diffuse alveolar hemorrhage (38%). Non-invasive techniques led to the diagnosis in 38% of the episodes. The diagnostic yield of blood culture was 16%, of sputum cultures 24%, of NPW 15%, and of TBAS 29%. Bronchoscopic techniques were diagnostic in 53% of the episodes in which they were employed (BAL 44% and PSB 16%). Antibiotic treatment was changed according to the results obtained with the different techniques in 48% episodes. Overall mortality was 52.5%, this being higher in those patients with an infectious etiology (64%) as compared to those with a non-infectious etiology (28%) (p: 0.05) and also in those patients requiring mechanical ventilation (97%) in comparison to those not requiring it (10%) (p <0.0001). In the univariate analysis the following variables were related to a poor prognosis: APACHE II score >20, PaO_2/FiO_2 <250, bilateral radiographic involvement and mechanical ventilation. Although the diagnostic yield of PI has improved with this approach, allowing an etiology-adjusted treatment, the mortality in this population is still too high.

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ELIMINATION OF NON-HODGKIN'S LYMPHOMA (NHL) AND CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) WITH A RHODAMINE-DERIVED PHOTODYNAMIC THERAPY

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TH9402 is a dibromorhodamine derivative with potent photosensitizing capacity that targets malignant cells through specific intracellular handling and mitochondrial localization, leading to the demise of targeted cells via oxydative damage. The importance of mitochondrial involvement in anti-apoptotic regulatory pathways of several NHLs prompted us to evaluate TH9402 photodynamic therapy for the elimination of B-lineage lymphoma and leukemia. The influx/efflux kinetics and cytotoxicity profile of TH9402 were evaluated against B-lineage cell lines (Raji, Namalwa, DHL16 and RL), normal B lymphocytes, bcl-2/IgH rearranged patient NHL cells, and primary B-CLL cells. Retention of TH9402, as measured at rest by HPLC, and upon light excitation by flow-cytometry, was maximum after 40 minutes. Cytotoxicity towards cell lines, as measured in a limiting dilution assay, was directly proportional to the concentration of TH9402, cellular concentration and light energy applied; and inversely proportional to the time of dye efflux from cells, achieving more than 5 logs of eradication of Raji, Namalwa, DHL16 and RL cells. Elimination of normal B lymphocytes, as well as patient NHL and CLL cells were evaluated after culture on a CD40 ligand expressing feeder layer. Normal (n=6) and malignant B-NHL (n=5) and CLL (n=4) cells were eradicated according to dose-intensity parameters, TH9402 achieving 2.5 or more logs of cytotoxic activity (the detection threshold of the assay). Moreover, in all NHL samples, bcl-2/IgH rearranged cells were not detectable, using a highly sensitive nested PCR assay, after PDT with TH9402. Importantly, treatment of normal hematopoietic progenitors, in the same conditions, preserved greater than 40% of CFU-GM, BFU-E and CFU-Mix colonies, and >75% of LTC-IC. In conclusion, photodynamic therapy with TH9402 can eliminate several logs of B-lineage NHL and CLL cells while preserving normal progenitors for hematopoietic engraftment. A clinical trial will evaluate this phototherapeutic approach for the elimination of B-lineage cells prior to autologous transplantation.

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DEFINITIVE TREATMENT OF ADVANCED MALIGNANT DISEASE BY ADOPTIVE IMMUNITY

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Twenty-one patients with advanced or chemotherapy resistant malignancies (including 6 AML, 3 ALL, 2 MDS, 6 NHL, 2 Acute Phase CML, 1 Myeloma, 1 Renal Cell) were subjected to allogeneic stem cell transplantation from an HLA compatible sibling (19) or unrelated donor (2) under subablative conditioning. All of these patients were relapsed and failures on chemotherapy They were also not candidates for ablative bone marrow transplantation because of age > 60 years (11), infirmity (6) or failure of previous allogeneic BMT (4). Patients were conditioned by Fludarabine, anti-thymocyte globulin and subablative doses of Cyclophosphamide (for lymphoproliferative) or Busulfan and VP-16 (for myeloproliferative disease). After infusion of > 5 x 10^6 CD34+ and > 10^8 CD3+ cells/kg, patients were started on cyclosporine (CSP) to prevent GVHD. In the absence of GVHD at +30 days, CSP was discontinued and 10^8 CD3+ cells/kg were infused as a boost. Regimen related toxicity was mild and well tolerated particularly in older patients. Graft versus host disease was the main cause of therapeutic failure and death. Persistent and fatal malignant disease was seen in only 2 AML and 1 ALL patients. Nine patients are alive and free of disease from 1-53 months with a projected survival of 25%. The survivors include 5 patients with leukemia, one patient with lymphoma and one with renal cell carcinoma. In all of the survivors the complete engraftment was associated with disappearance of bulk disease. One 12-year-old girl with AML,

having relapsed post-allogeneic BMT, was transplanted 9 times over 4 years to maintain her in CR and full donor engraftment. She was conditioned by mitoxantrone, HIDAC and VP-16 with no GVHD prophylaxis. Therefore it appears that these allografts were therapeutically competent and could achieve remissions when chemotherapy failed and that these remissions could be durable.

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ONCE DAILY INTRAVENOUS BUSULFAN (IVBU): PHARMACOKINETIC STUDY AND COMPARISON WITH ORAL BUSULFAN (POBU) IN COMBINATION WITH FLUDARABINE (FLU) AS CONDITIONING FOR ALLOGENEIC STEM CELL TRANSPLANT

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Myeloablative conditioning comprising FLU 50mg/m^2 on days -6 to -2 plus BU(Busulfex®) on days -5 to -2 inclusive was given to 75 adults. Twenty-five received POBU l mg/kg QID x 16 doses and 50 received IVBU 3.2 mg/kg over 3 hours daily x 4. Blood specimens were collected on the 1st and 4th doses to determine the disposition of IVBU when given once-daily. The IVBU and POBU groups respectively included similar proportions of patients with high-risk malignancy (66% vs. 67%), unrelated or genotypically mismatched-related donors (34% vs 26%) and recipients of bone marrow rather than blood (46% vs 44%). All patients received acute GVHD prophylaxis with CsA plus methotrexate and folinic acid. 98% IVBU and 60% POBU also received ATG (Thymoglobulin, Sangstat) 4.5mg/kg over 3 days pretransplant ($p < 0.0001$). IVBU patients developed significantly less grade 2 stomatitis (60% vs 96%, $p = 0.0008$). Hemorrhagic cystitis occurred in 10% IVBU and 8% POBU. Elevations of ALT and bilirubin were similar in the month post-transplant. One case of reversible VOD occurred, after POBU. There were two graft failures (one in each group) in recipients of unrelated donor marrow. There were 2 transplant-related deaths (4%) by 100 days in the IVBU group (from myocardial infarction and PTLD) and one (from acute GVHD) in the POBU group (4%). Preliminary PK mean parameters from 6 patients are summarized in the table. IVBU continues to display linear PK with expected exposure (AUC) four times that observed with the standard Q6H regimen, with no accumulation. Daily IVBU seems as well tolerated as POBU, has presented no unexpected toxicities, and is more convenient than QID dosing.

BU dose # (mg/kg)	$T_{1/2}$ (hours)	Cmax(mcg/ml)	Clearance(ml/min/kg)	AUC(μM*min)
#1 (2.98 ± 0.2)	2.5 ± 0.2	3.9 ± 0.3	2.5 ± 0.4	4952 ± 791
#4 (2.98 ± 0.2)	2.6 ± 0.4	4.0 ± 0.3	2.5 ± 0.5	4980 ± 883

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GRAFT RELATED SURVIVAL FOLLOWING ALLOGENEIC PERIPHERAL BLOOD HEMATOPOIETIC CELL TRANSPLANTATION WITH TACROLIMUS AND METHOTREXATE GVHD PROPHYLAXIS

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We assessed the benefit of tacrolimus GVHD prophylaxis in allogeneic peripheral blood hematopoietic cell transplantation. Forty-nine patients with hematologic malignancies were entered on study. Donors were mobilized with G-CSF 16 μg/kg/day x 5 with apheresis starting on the fifth day processing 20 l to collect a minimum of 2x10^6 CD34$^+$ cells/kg recipient weight. All 49 have engrafted without graft failure. The first 14 patients received tacrolimus alone for GVHD prophylaxis. Four patients remain alive and in remission 1207 to 1439 days following transplantation. Two patients died of relapsed disease. Eight died of transplant related causes. The protocol was amended to alter GVHD prophylaxis by adding methotrexate 5 mg/m^2 days +1, +3, +6 and +11. 35 additional patients have been treated. Fifteen remain alive and in remission while an additional 4 patients remain alive with relapsed disease. Seven patients have died of relapsed disease. Nine patients have died of transplant related causes. Median follow-up to date is 448 days, ranging from 22 to 1439 days. Graft related toxicity was assessed by determining survival, censoring for deaths from relapsed disease. The 100 day, 1 year and 2 year graft related survival for patients treated without methotrexate is 71%, 57% and 46% and with methotrexate 80%, 74% and 74% respectively ($p=0.04$). There has been a marked improvement in outcome since the addition of methotrexate to the regimen with a decrease in severity of both acute and chronic graft versus host. The addition of short course methotrexate appears to impact intermediate and long term survival related to tacrolimus prophylaxis in allogeneic peripheral blood hematopoietic cell transplantation.

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COMPARISON OF HARVEST YIELDS OF PERIPHERAL BLOOD HEMATOPOIETIC CELLS FROM ALLOGENEIC AND AUTOLOGOUS TRANSPLANT DONORS

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We compared yields for hematopoietic cells from allogeneic (ALLO) and autologous (AUTO) donors. 60 ALLO collections were mobilized with G-CSF 16 μg/kg/day x 5 with apheresis on the fifth day, processing 20 l. 425 AUTO collections were mobilized with myelosuppressive chemotherapy and G-CSF 5 μg/kg/day continued until recovery from myelosuppression and completion of harvesting, processing 15 l. The target for collection was 2x10^6 CD34$^+$ cells/kg to 10^7 CD34$^+$ cells/kg. Circulating CD34$^+$ cells were comparable despite different mobilization approaches. Hematopoietic progenitor distribution was identical for the two groups. Difference in yield may reflect the different volumes processed. The level and distribution of hematopoietic progenitor cells achieved in allogeneic and autologous donors appear to be independent of the method of mobilization employed.

	ALLO	AUTO
Baseline		
Blood volume(BV ml)	4866±1078	4519 ± 1020
WBC/ml	7.7±2.8 x 10^6	5.8 ± 2.1 x 10^6
CD34$^+$ cells (%)	0.02 ± 0.03	0.01 ± 0.01
CD34$^+$ cells/BV	6.0±7.2 x 10^6	2.1±1.9 x 10^6
PB at collection		
WBC/ml	4.7±1.5 x 10^7	1.7 ± 1.3 x 10^7
CD34$^+$ cells (%)	0.09 ± 0.07	0.32 ± 0.72
CD34$^+$ cells/BV	1.9±1.5 x 10^8	1.9±3.9 x 10^8
CD34$^+$ fold increase	32	91
Apheresis		
Mononuclear cells/kg	7.4±3.7 x 10^8	1.9 ± 1.3 x 10^8
CD34$^+$ cells/kg	3.8±2.8 x 10^6	2.7±5.3 x 10^6
Total colonies/kg	5.9 ± 4.5 x 10^6	2.0 ± 2.4 x 10^6
% CFU–GM	34 ± 9	32 ± 10
% BFU–E	64 ± 9	67 ± 11
% CFU–GEMM	2 ± 2	2 ± 2

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LOW RISK FOR INFECTIOUS COMPLICATIONS AFTER BLOOD STEM CELL AUTOGRAFTING FOR MULTIPLE MYELOMA

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In multiple myeloma (MM), treatment with repeated high-dose Melphalan followed by blood stem cell autografting results in prolonged overall survival when compared to conventional chemotherapy. Neutropenic febrile complications are the main

transplant-related toxicity. To evaluate the impact of post-transplant infections in MM patients we compared data of 55 transplants for MM with data of 105 transplants for other diseases (NHL/HD 59, acute leukemia 10, GCC/BC 26, STS 10). All patients received prophylaxis with Ciprofloxacin/Fluconazole and G-CSF. Overall incidence of neutropenic fever (NF) was 65%. While the incidence of NF in MM was 42% without serious sequelae, the incidence of NF after transplant for other diseases was as follows: NHL/HD 75%, acute leukemia 80%, GCC/BC 77%, STS 100%. Duration of fever, days with antibiotic treatment and duration of hospitalization was shorter in MM patients. There was one sepsis-related death in a patient with NHL. The lower incidence of NF in MM correlates with a higher stem cell mobilization capacity, reinfusion of higher CD34 numbers, less mucositis and the use of non-TBI containing regimens. In conclusion, the risk of post-transplant infections in MM patients is considerably low. Therefore, first, blood stem cell autografting in multiple myeloma may be performed in an out-patient setting and second, should be considered as standard treatment also in elderly MM patients.

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DONOR T-CELL–DERIVED TNF IS REQUIRED FOR GVHD, GVL AND COMPLETE T-CELL CHIMERISM

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Previous studies in murine bone marrow transplant (BMT) models using neutralizing anti-tumor-necrosis-factor (TNF) antibodies or TNF receptor-deficient recipients have demonstrated that TNF can be involved in both graft-versus-host disease (GvHD) and graft-versus-leukemia (GvL). TNF in these GvHD and GvL models was thought to be produced by activated monocytes and macrophages. We used TNF-/- mice to study the specific role of TNF produced by donor T cells in a well established parent-into-F1 hybrid model (C57BL/6 → B6C3F1). Recipients of donor bone marrow + TNF-/- T cells developed significantly less morbidity and mortality from GvHD than recipients of wild type (wt) T cells. Thymic cellularity was significantly increased in recipients of TNF -/- cells, indicating a role for donor T cell-derived TNF in thymic GvHD. Other GVHD target organs (liver, intestines and skin) are currently being analyzed. In vitro proliferation to alloantigen as well as in vivo expansion of TNF -/- T cells was intact. Recipients of TNF -/- T cells that were also inoculated with 32Dp210 leukemia cells at the time of BMT showed increased mortality from leukemia when compared with recipients of TNF wt cells. Interestingly, we found that animals transplanted with TNF -/- T cells exhibited mixed chimerism with significant numbers of remaining host T cells on days 14 and 28 post-transplant, while recipients of wt T cells (as well as FasL deficient and perforin -/- cells) had virtually 100% donor T cell engraftment. This finding suggests a specific role for donor T cell-derived TNF in T cell chimerism and the elimination of host T cells, that has not been described previously. Further experiments in different murine models previously established to study graft rejection and engraftment are currently underway.

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ACCEPTABLE OUTCOME AFTER MAJOR ONE ANTIGEN MISMATCHED RELATED OR UNRELATED DONOR STEM CELL TRANSPLANTATION FOR ADVANCED LEUKEMIA

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Some patients with advanced leukemia do not have HLA-matched donors, therefore we offered selected patients allogeneic SCT from donors with single HLA-A, -B or -DR mismatches. Many donor/recipient pairs were also mismatched for HLA-Cw (n=6) or -DQ (n=4). We transplanted 17 patients using HLA-A (n=5), -B (n=3), -DR (n=9) mismatch-related (n=9) or unrelated (n=8) donors: 12 males; 5 females. Median age: 35 (19-54) yrs. All had advanced leukemia: AML>CR1 (n=6); untreated 2°AML (n=1); ALL>CR1 (n=5); CML>CP1 (n=5). Either bone marrow (n=9), PBSCs (n=7) or both (n=1) were used. TBI (n=14) or chemotherapy alone (n=3) were used as conditioning regimens. All patients received CSA/MTX plus 5 (related) or 10 days (unrelated) of ATGAM. Two patients died before engraftment. All others engrafted without early or late rejection. Fifteen patients were assessable for acute GVHD: grade 0 (n=2; 13%); grade I (n=3; 20%); grade II (n=7; 47%); grade III (n=1; 7%); grade IV (n=2; 13%). Nine (53%) patients died before day +100: multiorgan failure (n=2); acute GVHD (n=2); interstitial pneumonitis (n=2); infection (n=1); relapse (n=2). Eight patients were assessable for chronic GVHD: none (n=4; 50%); limited (n=2; 25%); extensive (n=2; 25%). TRM was 7 (41%) of 17 patients, and relapse occurred in 4 (40%) of the remaining 10 patients. There were 2 deaths >day +100, both due to relapse leaving 6 (35%) patients surviving at a median of 391 (112-919) days. Of survivors, 2 had HLA-A, 1 -B, 3 -DR mismatches. Three remain on tapering doses of immunosuppression at +112, +125 and +433 days with Karnofsky scores of 80-90%; and 3 are on no medications at +349, +581 and +919 days with Karnofsky scores of 100%. Our results show that selected patients with advanced leukemia can be successfully treated with SCT using related or unrelated donors with a major HLA mismatch.

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AN AUDIT OF A PCR-BASED CMV SURVEILLANCE STRATEGY FOR PATIENTS UNDERGOING ALLOGENEIC STEM CELL TRANSPLANTS

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CMV reactivation is a major complication in at-risk patients undergoing allograft. Prophylactic strategies utilising Ganciclovir in all at-risk patients have been shown to reduce the incidence of CMV reactivation, but result in many patients being treated unnecessarily. With the development of sensitive PCR and antigenaemia assays designed to detect CMV reactivation before the development of clinical disease, strategies of intensive surveillance, with preemptive therapy at the first sign of reactivation have been implemented. We sought to audit our CMV surveillance strategy - to identify organisational problems, assess the CMV reactivation rate, and monitor the outcome of preemptive therapy. All allografts performed at our centre over a 14-month period were included. At-risk patients received high-dose aciclovir prophylaxis. CMV surveillance was performed weekly on plasma samples using 2 PCR detections; testing was continued at least to day +105. Patients with positive results were treated with GCV twice daily for 2 weeks and then once daily, 5x weekly, with treatment monitored by PCR assays. Nineteen sibling allografts were performed during the study period of which 14 were CMV at-risk. Audit of testing frequency showed that a PCR result was obtained in 108/132 (82%) possible weeks. Reasons results were not obtained included wrong sample type, transportation delays, and laboratory failure. PCR result became positive in 4/14 patients. In 3 cases, CMV was cultured on shell-vial assay from the urine 0, 7 and 20 days from the time of PCR positivity. Treatment with ganciclovir was effective in all cases. We conclude that audit is essential to identify organisational problems with a CMV surveillance policy. We report a low incidence of CMV reactivation in our patients. We show that the "lead-time" from positive PCR result to CMV disease may be short, emphasising the need to optimise organisational issues, and start treatment at first PCR positivity.

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RISK FACTORS AND CLINICAL CHARACTERISTICS OF HEPATITIS B VIRUS (HBV) REACTIVATION POST ALLOGENEIC STEM CELL TRANSPLANTATION (ASCT) IN PATIENTS PREVIOUSLY INFECTED AND IMMUNE TO HBV

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The immune deficiency associated with aSCT can compromise the effector function of HBV specific CD8+ cells and contribute

to HBV replication posttransplant. Moreover, corticosteroids used for GVHD prophylaxis/treatment may contribute to further accumulation of HBV peptides on the hepatocyte surface through direct genomic transcriptional regulation. Immune reconstitution and immunosuppressive therapy withdrawal can then lead to clinical flare-up. To identify risk factors and clinical characteristics associated with HBV reactivation post aSCT in patients immune to HBV, KFSH&RC IBMTR database from January 98 to June 2000 was reviewed. Of 131 patients treated with aSCT, 127 had complete screening for HBV. Fifty-three (42%) had prior infection to HBV and were immune (HBsAg-, HBeAg-, HBcAb+, HBsAb +/-). Treatment related deaths before day 120 occured in eleven patients,and eight were on full immunosuppressive therapy at the time of analysis. Thirty-four were evaluable. Five (15%) reactivated, and developed hepatitis with seroconversion (HBsAg+, HBeAg+) and/or +HBV DNA by PCR. Three groups were identified. I: 22 patients with no GVHD. One/22 (4.5%) reactivated at 5.5 months post aSCT. II: 5 patients with aGVHD responsive to therapy. No reactivation. III: 7 patients with cGVHD, where 4 (57%) reactivated at 18, 18, 19, and 21 months post aSCT. Three patients had clinical evidence of cGVHD at the time of reactivation. Only 1/27 patients without cGVHD had HBV reactivation, in comparison to 4/7 with cGVHD, p=0.0035, relative risk 15.4 (CI 95% 2.0-117). Reactivation occurred during immunosuppressive therapy tapering in two patients and at 20, 25, and 40 days after its withdrawal in three. Flare up resolved in four patients within a period of 10 weeks after initiation of anti-viral therapy, while one died of pulmonary infection during flare up. Prospective molecular monitoring of patients at risk could lead to early diagnosis of HBV reactivation and timely institution of anti-viral therapy.

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IMMUNOTHERAPY WITH DONOR LEUKOCYTE INFUSIONS FOR TREATING CHILDREN WITH POST-TRANSPLANT RELAPSED ACUTE LEUKEMIA: SINGLE CENTER PEDIATRIC EXPERIENCE

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Donor Leukocyte Infusions (DLIs) offer a therapeutic option for patients with post allo-BMT relapse,though its efficacy in acute leukemia is questioned. We reviewed our Institutional DLI experience for children with post-transplant relapsed leukemia. Between 1997 and 2000, 9 children (5 ALL,4 ANNL) received DLI from HLA-matched family donors. Median interval between relapse and BMT is 7 months (Range: 2 to 22 months). We used filgrastim (10 mcg/kg/day x 5 days) mobilised peripheral blood stem cells as a source of donor leukocytes. All received cytoreductive chemotherapy prior to DLI:7 received 2-chlorodeoxyacetate(10 mg/m^2 on Day 1-3)and Idarubicin (8 mg/m^2 on day 1-5), 2 ALL children re-induced with salvage protocols. First course of DLI was given at a median of 25 days (12 to 67 days) from initial relapse: 5 received 1 infusion,4 received 2 infusions. Median CD3 count for the first infusion was $1.6x10^8$ cells/kg (1 to 9); for the second infusion $4.6x10^8$ cells/kg (1.3 to 8.9). Corresponding CD34+ counts: $1.8x10^6$/kg (0.9 to 9.6) and $5.7x10^6$/kg (1.3 to 5.7) respectively. Among 5 ALL children: 1 died with extensive cGVHD (15 months), 1 died with postDLI pancytopenia related complications(4 months), 2 relapsed at extramedullary sites (1 testis, 1 breast at 4 and 25 months respectively): both alive with disease. Among 4 ANLL:1 relapsed (limited cGVHD)and died, 1 with isolated testicular relapsed at 24 months is alive 2 years after second allo-BMT,2 alive in CR with extensive cGVHD at 26 and 43 months (Lansky scores 60 and 80%). In summary, 7 children responded to DLI:4/7 transient response(2 ALL,2 ANLL),3/7 durable response(1 ALL,2 ANLL);all developed GVHD. Median follow up 1.8 years(Range 4 to 43 mths). Overall actuarial survival :56± 17%. EFS at 2 years 22± 14%. This limited sample shows cGVHD significantly affects EFS (p=0.02). The likelihood of inducing remisson appears better for ANLL. More insight is required to offer the benifit of GVL effect without inducing GVHD morbidity.

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PHASE I DOSE ESCALATION STUDY OF MELPHALAN WITH IFOSFAMIDE, CARBOPLATINUM, AND ETOPOSIDE (MICE) WITH AUTOLOGOUS STEM CELL SUPPORT

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High dose ifosfamide, carboplatinum, and etoposide have been studied extensively and shown to be effective in a wide range of malignancies. The addition of another alkylator in high doses may potentiate the activity of this regimen. We devised a phase I dose escalation study of melphalan in combination with fixed doses of ifosfamide, carboplatinum, and etoposide (MICE) followed by autologous stem cell rescue. Six patients were entered on study, 4 males, with a median age of 26 (21-48) years. All patients had failed standard therapy and had measurable disease. The median number of infused CD34+ cells was 2.6 x 10^6/kg (0.8 -5.9x 10^6/kg). The preparative regimen consisted of Ifosfamide 14gm/m^2 divided into 8 doses given IV bolus q 12 hours with MESNA in equivalent doses given by CIV D-6 through D-3, carboplatinum AUC 6 IV bolus q day for 4 doses D-6 to D-3, and etoposide 1600mg/m^2 divided into 8 doses IV q 12 hours D-6 to D-3. The first dose level of melphalan was 60mg/m^2 divided into two equal doses on D-8 and D-7. All patients received only the first dose level of melphalan. One patient developed grade V lung toxicity, although it was unclear if this was interstitial pnemonitis or tumor progression, and another developed grade III renal failure with grade IV elevation of creatinine. Three patients have died, all with tumor progression: 2 germ cell cancers and 1 soft tissue sarcoma. Three patients survive: one with Ewings sarcoma in CR, one with phyloides sarcoma and disease progression, and one with small cell lung cancer that is too early to assess. In conclusion, we believe this regimen has excessive toxicity and do not recommend its use at these dose levels. We plan to study a similar combination of drugs with less ifosfamide and further dose escalations of melphalan.

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INFECTIONS IN PATIENTS WITH HIGH RISK MALIGNANCIES UNDERGOING THERAPY WITH A NONMYELOABLATIVE REGIMEN FOLLOWED BY ALLOGENEIC STEM CELL TRANSPLANTATION

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Less intensive regimens followed by allogeneic stem cell transplantation have been increasingly used. Few data are available about infectious complications after these regimens. Here we report our data on 12 patients (pts) with high risk malignancies treated with fludarabine (25 mg/m^2x3) doses and cyclophosphamide (750mg/m^2x3 doses) followed by allogeneic peripheral blood stem cell transplantation from HLA identical siblings. In addition 4 pts received antithymocyte globulin at 30mg/kg x 3 doses. As GVHD prophylaxis 9 pts received cyclosporin (CSA) and minidose methotrexate, 2 pts CSA and mycophenolate mofetil and 1pt CSA and prednisone. CSA was given until day 60 then tapered if no GVHD occured. All pts received low dose amphotericin as antifungal prophylaxis, CMV and PCP prophylaxis. There were 5 females and 7 males with a median age of 57 years (range 31 to 67). Five pts had previous transplants (2 auto, 3 allo). All pts were CMV positive with 10 of 12 donors also positive. G-CSF mobilized blood stem cells were infused with a median of $5x10^6$/Kg CD34+ and $2x10^8$/Kg CD3+. Eleven pts engrafted with a median time to ANC $\geq 0.5x10^9$/L of 11 days (range 6 to 13)and a median time to platelet $\geq 20x10^9$/L of 14 days (range 6 to 18). No bacterial infections were diagnosed during the neutropenic period. CMV reactivation was diagnosed in 8 pts, presumed aspergillus infection in 1 pt and proven aspergillus in 2. Two pts developed adenovirus enteritis and 1 HSV enteritis. The two pts developing adenovirus enteritis were treated with cidafovir with resolution. Seven pts had grade III-IV acute GVHD. Aspergillus infection contributed to the death of 2 pts. In Summary: Although the number of pts in this series is small, a less intensive conditioning regimen does not lower the incidence of fungal or viral infections in this poor risk population.

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METACHROMATIC LEUKODYSTROPHY (MLD) TREATED WITH VUD BMT. A NEW THERAPY FOR A PREVIOUSLY LETHAL DISORDER

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MLD is a lethal autosomal recessive disease, caused by the inherited deficiency of arylsulphatase A. Deficiency of this enzyme causes intralysosomal storage of the sphingolipid cerebroside sulphate. Patients suffer from a progressive demyelination in both central and peripheral nervous system. The disease is invariably fatal. BMT provides a method for permanently replacing deficient enzyme activity. Microglia and astraglia are derived from the newly engrafted marrow and corrects the recipient's enzyme deficiency. There is only one published report of allogeneic related transplantation in an adult patient with MLD. The patient had disease stabilization for 4 years, but no improvement. We report a 22-year-old man who underwent BMT for MLD. He presented with numbness of lower extremities and paraparesis. Within 4 years, he developed dysarthria and became wheelchair-bound. EMG studies showed severe demyelinative neuropathy. MRI of the brain showed bilateral symmetric white matter disease. The diagnosis of MLD was confirmed by a low arylsulphatase A level in leukocytes. He had no siblings but an HLA-matched unrelated donor was identified through the NMDP. His conditioning regimen included cyclophosphamide and 1500 cGY of TBI. He engrafted and had 99.7% gender mismatched donor mononuclear cells by day 23 on FISH analysis. He had normal leukocyte arylsulphatase A level by 3 months and a MRI done at 5 months showed resolution of earlier white matter disease. EMG studies showed improvement. His dysarthria resolved and he is walking. This report highlights the potential cure of this lethal disorder by repopulation the glial cells of the nervous system. Reports in other storage diseases suggest limited benefit of BMT, in the presence of neurological compromise. Our results may be superior due to the use of an unrelated instead of a sibling donor.

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HIGHER DOSE TOTAL BODY IRRADIATION WITH ALLOGENEIC BMT FOR CHRONIC PHASE CML RESULTS IN LOW RELAPSE RATE

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The most common conditioning regimens for allogeneic BMT in CML are TBI/CY or Bu/CY. Ideal doses of TBI have not been determined. We describe 44 patients with CP-CML undergoing sibling HLA–matched BMT using TBI and CY. Median age was 37 years (range, 12-63 years) with 61% males and 39% females. Preparative regimen was TBI 1500 cGy, 10 fractions of 150 cGy d–8 to –4, CY 60 mg/kg QD d–3 and –2. BMT was unmanipulated. GVHD prophylaxis included CSA or Tacrolimus and MTX. Supportive care included gut decontamination, sterile LAF, fluconazole, acyclovir and PCP prophylaxis. No growth factor support was used. Median follow-up for this cohort is 603 days post-BMT (range, 19-3657 days). Twenty-one transplants were sex-mismatched. Median time to an ANC of 500/μL was 23.5 days (range, 10-47 days), and to an ANC of 1000/μL, 26.5 days (range, 11-53 days). Median time to PLT >20,000/μL was 20 days (range, 10-47 days). Six patients died from infections and 5 from non-infectious causes. Acute GVHD occurred in 13 patients with 1 death. Four pts (9%) relapsed, 2 received DLI and one a second allogeneic transplantation. Overall transplant related mortality at day 100 was 27%. Twenty-eight patients remain alive on days 247-3788 post-BMT. Kaplan-Meier estimates for EFS at 3 years is 62±7%. Reported relapse rates post-HLA identical sibling transplant for CML-CP range from 19% with 1,200 cGY dose (Cliff et al, Blood, 77(8) 1991) to 30% with 1,000cGY of TBI (Devergie et al., Blood 85(8) 1995). This data suggest that a higher dose of TBI (1500cGY) results in a lower relapse rate although it results in significant TRM.

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SALVAGE OF A TUNNELED CENTRAL VENOUS ACCESS CATHETER WITH LONG TERM INTRAVENOUS LINEZOLID DESPITE VANCOMYCIN RESISTANT ENTEROCOCCAL (VRE) SEPSIS

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Vancomycin-resistant enterococcus is an increasing problem in hospitalized patients. Linezolid, a recently approved oxazolidinone, has demonstrated efficacy in the treatment of VRE. Recommended therapy generally includes the removal of any existing indwelling catheters. We are reporting a case in which a tunneled long-term central venous catheter was salvaged in an immunosuppressed BMT patient by administration of intravenous linezolid. The patient is a 49-year-old woman approximately 250 days status post-allogeneic–matched related-donor transplant for non-Hodgkin's lymphoma. Her transplant course has been complicated by extensive chronic graft versus host disease requiring significant doses of immunosuppressive medications. More recently, she developed pulmonary nocardiosis and an inferior vena cava tunneled catheter was placed for IV trimethoprim/sulfamethoxazole therapy. The inferior vena cava approach was her only option for long term access because multiple prior central venous devices limited catheter options. Approximately 1 month after line placement, she presented to clinic for routine evaluation with general malaise and a low grade fever. Blood cultures were drawn and vancomycin-resistant *Enterococcus faecium* was isolated from two of four blood cultures drawn from the catheter. Linezolid, 600 mg IVPB every twelve hours for 30 days was administered to the patient. Blood cultures drawn through the catheter five days after the initiation of therapy were negative. She was discharged and self-administered her remaining therapy as an outpatient. Blood cutures remain negative two months after discontinuation of therapy. Rectal swabs performed at 2 weeks and again at 2 months of therapy were negative for VRE colonization. The central access catheter is still in place and in intermittent use. In summary, while removal of potentially contaminated venous access in the setting of VRE is ideal, indwelling catheters may be salvaged with extended linezolid therapy.

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CULTURED DENDRITIC CELLS CAN PRIME POTENT CTL RESPONSES TO A HER-2+VE BREAST CANCER CELL LINE

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Dendritic cells (DC) are potent antigen-presenting cells, which are integral to the initiation of T-cell immunity. The ability to culture these cells in vitro has allowed DC immunotherapy to be investigated as a mechanism to enhance immune responses for a variety of malignancies. We want to establish an immunotherapy program for patients after high dose chemotherapy. This is the time when a patient is likely to have minimal residual disease, and when specifically targeted immune cells may eradicate remaining cancer cells. We undertook the following study to determine if we could induce CTL responses to breast cancer cells. Since many patients with metastatic breast cancer overexpress the HER-2 protein we have employed a HER-2 +ve breast cancer cell line as our antigen source. A sample of apheresis product was obtained at the time of apheresis from consenting patients, or from discarded frozen product. Dendritic cells were cultured from adherent PBMC in the presence of IL-4 and GM-CSF. After 7 days, dendritic cells were harvested and pulsed with tumor lysate. After a further 7 days, the primed cells were harvested, counted, and used in an LDH-based cytotoxicity assay. We could generate cytotoxicity (62-100%) at a high (10:1) effector (primed cells) to target cell (SKBR3) ratio. However, cytotoxicity dropped off when the E:T ratio fell to 5:1 (<15% cytotoxicity). By utilizing whole protein instead of lysate we were able to obtain up to 100% (23-100%) cytotoxicity at this lower E:T ratio of 5:1. Using total RNA resulted in 24% cytotoxicity at an E:T ratio of 0.5:1. Experiments are now focused on further purifying the antigenic source to reduce the E:T ratio, while ensuring a high level of specificity. Together this demonstrates that we can generate potent cytotoxic

responses to HER-2+ve breast cancer cells. Further experiments are underway .

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PREDICTIVE VALUE OF EARLY STUDIES OF IMMUNE RECONSTITUTION IN CHILDREN POST-UNRELATED CORD BLOOD TRANSPLANTS ON INFECTIOUS COMPLICATIONS AND ACUTE GVHD

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Objectives: Opportunistic infections are the major cause of death after transplantation with HLA-mismatched banked umbilical cord blood. Almost 80% of these infections occur during the first 100 days. We hypothesized that early studies of immune function will identify patients at increased risk for infections and those at risk for acute GVHD. Peripheral blood from 20 children at a mean of 51.4 days post-transplant was studied by 4 color surface-intracellular FACS and proliferation assays.

Results. All patients engrafted, and 14 of the 20 patients are alive, with a mean survival of 305 days. Seven of the 20 patients developed de novo opportunistic infections (6 viral, 1 fungal) and 12 developed grade II-IV aGVHD. Employing Spearman correlation analysis, a significant inverse correlation was found between age and recovery of T cells (p=0.006) and CD4+ T cells (p=0.0001). Unlike following bone marrow allografts, recipients of cord blood reconstituted T cells with a dominant CD4+ phenotype (mean: 62%). However, only 7% of T cells had the phenotype of recent thymic emmigrants (CD45RA+/CD62L+). Mean PHA count was 25,832CPM. There was significant correlation between PHA responses and T cell (p=0.001) and CD4+ (p=0.007) cell recovery. Lymphoid DCs (1.4/μL) and myeloid DCs (16.4/μL) were detected. We found significantly lower (*t*-test) CD3+ (p=0.04) and CD4+ (p=0.001) counts in patients with opportunistic infections compared to ones without. Patients with infections demonstrated an increase of T cells expressing TCRγδ, CD8, and HLA-DR (p=0.001). The proportion of T cells secreting Th1-type cytokine was significantly elevated in patients with GVHD measuring mean % IL-2 at 3.7% vs 20.3%, TNFα: 1.5% vs 20.6%, IFNγ 2.5% vs 10.9% in the absence (n=6) vs presence (n=5) of grade 3-4 aGVHD respectively.

Conclusion: This analysis suggests that early monitoring of immune reconstitution will aid identification of patients at risk for development of opportunistic infections and acute GVHD.

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FACTORS AFFECTING NEUTROPHIL AND PLATELET RECONSTITUTION FOLLOWING T-CELL–DEPLETED BONE MARROW TRANSPLANTATION: DIFFERENTIAL EFFECTS OF GROWTH FACTOR TYPE AND ROLE OF CD34+ CELL DOSE

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Univariate and multivariate analyses were performed to identify the factors that affect the kinetics of neutrophil and platelet recovery in 546 recipients of T-cell–depleted marrow allografts. Marrow was depleted of CD3+ cells by complement-mediated lysis using T10B9 -1A3 (N=489) or Muromonab-Orthoclone OKT3 (N=57) mAb. Neutrophil engraftment to 0.5 x 10^9/L and platelet engraftment to 20 x 10^9/L were assessed as endpoints. Factors significantly affecting neutrophil or platelet engraftment in the univariate analysis included: patient age, T-cell dose, ATG, gender, diagnosis, CMV serostatus, HLA mismatch, CD34 cell dose (N=249), and growth factor use and type. These variables were included in the multivariate Cox proportional hazards regression model. The results showed that faster neutrophil engraftment was independently associated with CD34+ cell dose ≥ 5 x 10^6/kg and most strongly with growth factor. Faster platelet engraftment was associated with transplant for chronic leukemia, CD34+ cell dose ≥ 2 x 10^6/kg, an HLA matched related donor, and the absence of growth factor use. G-CSF had a higher relative risk (RR) of enhancing neutrophil engraftment and in delaying platelet engraftment than GM-CSF, and the combined use of G-CSF + GM-CSF was similar to G-CSF alone. The enhancing effect of G-CSF for neutrophil recovery was most striking for patients who engrafted to 0.5 x 10^9/L ≤ day 12 (RR=9.5, p=<0.0001) compared to patients who received no growth factor. Conversely, the delaying effect of G-CSF on platelet engraftment was strongest for patients engrafting ≤ 25 (RR=0.4, p=0.0004). Of the independent variables affecting engraftment kinetics only growth factor and to a limited extent, CD34+ cell dose can be controlled by the clinician. A higher CD34+ cell dose enhances the rate of both neutrophil and platelet engraftment whereas the benefits of myeloid growth factor in enhancing neutrophil recovery may be partly offset by a delay in platelet engraftment.

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STRATEGY TO FACILITATE PROSPECTIVE STUDY DESIGN AND CONDUCT

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Introduction: The quality of protocol design, selection of study methods and data collection process may influence the interest in and commitment to participation in prospective clinical studies.

Methods: Protocol design should start with identification of established prognostic factors and treatment procedures. A data analysis plan listing factors that define patient, treatment, graft, donor and outcome parameters should subsequently be drafted. Definition of primary and secondary outcome parameters is mandatory. Study objectives should in first instance reflect evaluation of safety and efficacy of the treatment/product that will be tested and should address outcome parameters. Selection of established treatment procedures in the study design to support evaluation of the therapy to be tested will facilitate study conduct. Limited data collection focussing on baseline patient characteristics, potentially prognostic factors, major complications, outcome parameters and on checking whether the study procedure was pursued in accordance with protocol should be sufficient to analyse the results of the study, to answer questions posed in the objectives and to identify new prognostic factors.

Presentation: Explanation of the study objectives, study design, study procedures and the data collection process will largely enhance comprehension of the study and will invite participation. Clear oral communication is important to create correct understanding. The author will present this approach from educational perspective.

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EX VIVO TREATMENT OF ALLOGENEIC LYMPHOCYTES WITH L-LEUCYL-L-LEUCINE METHYL ESTER PREVENTS GRAFT-VERSUS-HOST DISEASE IN HAPLOIDENTICAL MURINE MODEL

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L-leucyl-L-leucine methyl ester (LLME) is a lysosomotropic agent that is taken up by cells through a saturable facilitated transport mechanism. Once intracellular, LLME is converted to pro-apoptotic (Leu-Leu)-OME metabolites through the acyl-transferase activity of dipeptydil-peptidase I (DPPI). LLME induces programmed cell death of DPPI-expressing NK cells, monocytes, granulocytes, the majority of CD8+ T cells, and a fraction of CD4+ T cells. Graft-versus-host disease (GVHD), a major complication of allogeneic hematopoietic stem cell transplantation, is mediated by contaminating donor T cells present in the transplant inoculum, which can also be beneficial in enhancing engraftment, in prevention of opportunistic infections, and in combating leukemic relapse. Delayed lymphocyte infusion (DLI) was implemented in an effort to balance some of these risk factors. In clinical trials, while DLI was quite effective in treating leukemic relapse, approximately one third of the patients still developed severe GVHD. LLME has been previously shown to prevent GVHD in animal conventional transplant models. In this study, we sought to investigate the use of LLME in DLI in a haploidentical murine model. LLME treated T cells were given as DLI two weeks after hematopoietic stem cell transplantation. The LLME

treated DLI resulted in reduced occurrence and severity of GVHD while maintaining a significant level of antileukemic activity. In an effort to further promote engraftment, antileukemic responses and reduce the length of the immunosuppression while preventing GVHD we transplanted the LLME treated lymphocytes together with the stem cells. The results clearly demonstrated that mice transplanted with LLME treated lymphocytes had a 100% survival as compared to those mice that received non LLME treated lymphocytes. Transplantation of LLME treated lymphocytes with stem cells can potentially provide a more targeted approach for preserving antileukemic activity and helping to stabilize engraftment while reducing the occurrence of GVHD.

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DONOR CONDITIONING TO REDUCE RISK OF ACUTE GRAFT VERSUS HOST DISEASE (AGVHD) USING PERIPHERAL BLOOD STEM CELLS (PBSC) FOR MATCHED RELATED DONOR (MRD) ALLOGENEIC BONE MARROW TRANSPLANTATION (ALLOBMT)

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From 2/98 through 5/00 59 adults (age 22-64, median 47) had MRD alloBMT for malignant diseases using PBSC. GVHD prophylaxis was CSA/Mtx with CSA/methylprednisolone substituted for renal dysfunction (N=8). Donors received 10-16 μGm/Kg of G-CSF for 4-5 days then large volume leukopheresis. Thirty-one control patients conditioned with BuCy(2) or BuCyVP (7/31) plus ATG received PBSC depleted of T-cells *ex vivo* using the CellPro device. Twenty-eight patients conditioned with BuFludara (cohort II) received PBSC not *ex vivo* T-cell depleted but from donors given dexamethasone 10 mg/M^2/dX3 beginning 2 days prior to the collection in addition to the G-CSF. Distribution of age, diagnosis, GVHD prophylaxis, and time to ANC > 500/μL were similar. Six controls and 4 cohort II patients were inevaluable for AGVHD due to early death (4) or withdrawal of GVHD prophylaxis to treat relapse (6). Controls received 2.2 to 23.4X10^5 (med - 7.4) CD3+ cells/Kg and cohort II received 6.3 to 70.8X10^7 (med - 24.5) CD3+ cells/Kg. Controls received 1.0 to 11.9X10^6 (med - 5.7) CD34+ cells/Kg, cohort II received 3.6 to 27.5X10^6 (med - 11.0) CD34+ cells/Kg. Within cohorts there was no correlation between number of CD3+ cells infused and AGVHD nor between the number of CD34+ cells infused and time to ANC > 500/μL. Overall grade 2+ AGVHD occurred in 6 of 25 (24%) evaluable controls and 7 of 24 (29%) evaluable cohort II patients. Two patients in cohort II died of grade IV AGVHD. In this study the AGVHD incidence and severity using dexamethasone-treated PBSC donors and a BuFludara preparative regimen was only slightly greater than that achieved with BuCy conditioning and *ex vivo* T-cell depleted PBSC despite 300 times the CD3 cell dose. The less toxic preparative regimen, removal of alloreactive T-cells by dexamethasone, and/or the higher CD34 cell dose may explain this result.

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VALIDATION OF A LIMITED SAMPLING STRATEGY (LSS) FOR PHARMACOKINETICALLY DIRECTED DOSING OF HIGH-DOSE INTRAVENOUS BUSULFAN (BUSULFEX) IN BMT PREPARATIVE REGIMENS

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Variation in the area under the concentration/time curve (AUC) for oral busulfan results in a substantial risk of over or under treatment with excess risk of toxicity or relapse. Busulfex use reduces this variability by eliminating variability in absorption. Variability due to drug metabolism remains, but simplified pharmacokinetic study may be employed to achieve a specific target AUC. Conventional sampling strategies for determining AUC after oral administration use 12 samples over six hours to assure accurate tracking of erratic absorption. With Busulfex there is no necessity for drawing plasma levels during the infusion since busulfan pharmacokinetics are well described by single compartment, 1st order elimination models. This eliminates the requirement for a second iv access to avoid error due to contamination of the sample with drug. In theory, only peak and trough levels should be necessary, but to assure reliability for clinical decision-making, outlier values must be able to be identified. This requires at least four samples. We collected 11 samples from 13 adult patients and compared the AUCs obtained using all samples versus only the 5 samples collected hourly after the end of the infusion. The mean AUC calculation was 5% higher (1002 vs 956 μM-min) when only the smaller number of post infusion samples were used and the CV was substantially better (4.6% vs 8.2%). We now have used 5 sample data to dose over 40 patients. To further validate this approach we will present an analysis of pharmacokinetic data from 98 patients in the Busulfex pivotal clinical trials. The AUCs based on all 11 samples from each patient will be compared to the AUC based on the 5 post infusion samples. We predict this will confirm comparable reliability, and possibly superior accuracy, of Busulfex AUC determination using 5-sample versus 11-sample testing while reducing laboratory and nursing costs.

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DECREASED INCIDENCE OF CMV VIREMIA AFTER ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTS

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CMV infection can be detected by PCR, antigen-based or culture methods. Using sensitive PCR, CMV viremia can be detected in up to 60% of Bone marrow transplant recipients by day 100 and in up to 75% when CMV seronegative donor-recipient pairs (CMV-/-) are excluded (Einsele et al Blood86:2815,1995). We evaluated the incidence of CMV viremia in 52 consecutive allogeneic stem cell transplant recipients using a sensitive PCR (8 CMV-/- patients were not included). 17 patients (pts) had lymphoma, 28 had leukemia, 2 had MDS, 2 had myelofibrosis, 1 had aplastic anemia and 2 pts had sickle cell disease. The median age was 46 (14-64). 48 patients received unmanipulated stem cells from a matched sibling, 3 from a matched unrelated donor and 1 received T-depleted stem cells from a haploidentical relative. Conditioning consisted of Fludarabine/thiotepa/TBI in 26 pts, BEAM in 14, Cy/TBI in 2 and fludarabine/melphalan in 18 patients (haplo-identical transplants and sickle cell patients also received ATG 40 mg/kg). GVHD prophylaxis consisted of tacrolimus and mini-methotrexate. No prophylactic ganciclovir was given, but CMV viremia was treated with ganciclovir BID. No cases of CMV pneumonia occurred, and the cumulative incidence of CMV viremia was 35% ± 14% by day 100 and 38% ±15% by day 150. Donor type, underlying disorder (leukemia vs lymphoma) and previous transplant were not associated with the risk for CMV viremia. The cumulative risk for CMV viremia by day 150 was 62% ± 25% in those receiving fludarabine/melphalan conditioning vs 27 ± 16% in pts receiving myeloablative regimens (P=0.019). In conclusion, the risk for CMV viremia after allogeneic PBSCT is considerably lower than after allo BMT. This may be related to more rapid immune reconstitution. Surprisingly, the risk for CMV viremia appears higher after non-myeloablative fludarabine/melphalan conditioning. This may indicate more profound immunosuppresion.

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AGNOGENIC MYELOID METAPLASIA WITH MYELOFIBROSIS CAN BE SUCCESSFULLY TREATED USING LOW INTENSITY CONDITIONING WITH FLUDARABINE AND MELPHALAN FOLLOWED BY ALLOGENEIC STEM CELL TRANSPLANTATION

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Agnogenic myeloid metaplasia (AMM) with myelofibrosis is a clonal hematopoietic disorder for which an allogeneic hematopoietic stem cell (HSC) transplant is potentially curative. Given the advanced age of most patients (pts) at diagnosis as well as the indolent nature of the disease in some, many pts are not considered candidates for standard myeloablative conditioning due to the anticipated toxicities. Here we report a series of

four consecutive pts at our institution with AMM who received an allogeneic HSC transplant following a low intensity regimen consisting of fludarabine (F) and melphalan (M). All pts had intermediate (N=3) or high (N=1) risk disease based on the criteria of Dupriez (Blood 88:1013, 1996). The median age was 56 years (range 48-58). Conditioning consisted of F (25mg/m^2 IV on days -6 to -2) and M (70mg/m^2 IV on days -3 and -2). All pts received G-CSF mobilized peripheral blood stem cells from HLA-identical siblings on day 0. The median CD34+ cell dose was 5.9 x 10^6/kg. All pts engrafted, achieving an ANC>1,000/μL on day +16 (range 12-20) and unsupported platelet count >20,000/μL on day +14 (range 11-28). All have achieved red blood cell transfusion independence. No pts experienced > grade 2 toxicity according to the criteria of Bearman. No pts developed grade II-IV acute GVHD. One of three evaluable pts has developed limited chronic GVHD. With a median follow-up of 140 days (range 60-320), all pts survive with a Karnofsky performance status of 100%, have achieved stable full donor chimerism, and have experienced documented regression of marrow fibrosis and splenomegaly. Although follow-up is short,these data are encouraging and suggest that a fludarabine and melphalan based conditioning regimen is relatively non-toxic yet promotes full donor chimerism in these pts. This regimen may expand the application of allogeneic HSC transplantation to older pts with AMM.

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CD8$^+$ T CELLS CONTROL THE EXPANSION OF γ/δ$^+$ T CELLS

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Using both murine and human sources (spleen, blood, BM, cord blood) we have previously reported conditions for the propagation of large numbers of CD3$^+$CD8$^+$TCRα/β$^+$ T cells which co-express NK cell markers and mediate antitumor activity. Such cells are generated by the timed addition of IFN-γ, α-CD3 and IL-2 followed by 14-21 days of culture. To investigate the role of the CD8 molecule in the recognition of tumor cells, we used splenocytes derived from CD8$^{-/-}$ mice and found that significant numbers of CD3$^+$CD4$^-$CD8$^-$TCRγ/δ$^+$ cells expanded (>300X expansion, ~100 x10^6/spleen). The expanded γ/δ T cells are morphologically large, heavily granulated, with foamy cytoplasm. They secrete significant quantities of IFN-γ, little TNF-α and no IL-4 or IL-10. Expanded γ/δ T cells mediate cytotoxicity against a variety of both syngeneic and allogeneic tumor cell lines. To confirm the role of CD8$^+$ cells in the inhibition of γ/δ T cells, we performed similar studies using splenocytes derived from either C57BL/6 or DBA mice. Depletion of CD8$^+$ T cells led to the expansion of large numbers of γ/δ T cells, indicating that CD8$^+$ T cells influence the homeostasis of γ/δ T cells. To establish whether CD8$^+$ cells inhibit γ/δ T cells through soluble mediators or cell surface molecules, transwell experiments were performed. These experiments showed that direct cell-to-cell contact is necessary for inhibition. Given this, we investigated whether the cytotoxic molecules FasL or perforin were responsible for CD8$^+$ mediated suppression of γ/δ T cells. Using purified CD8$^+$ cells derived from gld or pfp mice, we found that neither molecule was involved in the inhibition of γ/δ T cell growth. Taken together, these results show that large numbers of *ex vivo* expanded γ/δ T cells can be readily generated and that CD8$^+$ T cells control the expansion of γ/δ T cells through direct cellular interaction.

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A RANDOMISED, PROSPECTIVE COMPARISON OF ALLOGENEIC BONE MARROW AND PERIPHERAL BLOOD PROGENITOR CELL TRANSPLANTATION IN THE TREATMENT OF HAEMATOLOGICAL MALIGNANCIES

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We present the results of a prospective, randomised study comparing PBPC and BM focusing on engraftment, acute and chronic GVHD and survival. Sixty patients with haematological malignancies received HLA-identical sibling BM (group A) or PBPC (group B). Evaluable patients were 29 (A) and 27 (B). Median age was 36 (17-59) in A and 29.9 (7-51.5) in B. Conditioning regimen was mainly Bu-Cy2; GVHD prophylaxis was CSA-MTX. PBPC were harvested after 5 days of G-CSF 10mg/Kg/d. Median of infused CD34+ cells were 3.96x10^6 /kg (1.19-17.55) and 5.12 x10^6/kg (1.25-71.61) for groups A and B, respectively (P=0.32). Median of days for an ANC >0.5 x10^9 /L was 18 (13-30) in A and 15 (11-25) in B (P=0.02). Platelets >20 x10^9 /L occurred at +18 (10-40) in A and +12 (7-36) in B (P=0.001). Median days for discharge were 27 (18-69) and 21 (16-42) for groups A and B, respectively (P=0.01)The probability of ≥2 grade a-GVHD was 23% (A) and 26% (B) (P=0.53). The probability of extensive c-GVHD was 61% and 77% in A and B, respectively (P= 0.05), furthermore, all patientes in the PBPC group developed extensive disease, while 6/11 (54.5%) in the BM group (P= 0.01). The estimates of overall survival for A and B at 2000 days are 48% and 56% respectively (P=0.67); DFS at 2000 days are 50% and 60% respectively (P=0.47). PBPC resulted in faster neutrophils and platelets engraftment. The acute GVHD was similar in both groups, but the severity of c-GVHD was higher with PBPC. No differences in survival and DFS have been observed so far.

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HIGH RELATIVE RESPONSE OF MIXED LYMPHOCYTE CULTURE CAN PREDICT CHRONIC GRAFT-VERSUS-HOST DISEASE IN HLA-IDENTICAL SIBLING BMT

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The purpose of this study was the evaluation the mixed leukocyte culture (MLC) as an assay predictive of GVHD. From September 1993 to November 1999, 153 patients, 97 males, 56 females, median age 29 (3-59) years, were undergone BMT from HLA-identical siblings. Acute-GVHD was observed in 26 of 128 (20.3%) and chronic GVHD in 54 of 114 (47.4%) evaluable patients. For the analysis, one-way MLC assays were performed by standard method. Results of MLC were expressed using the relative response (RR) from donor against patient and ranged from -47.0 to 40.7% (median 0.50%). The probability of developing GVHD was determined for patients with positive and negative MLC. There was not significant difference in incidence of acute GVHD between the analysed groups. However, the incidence of chronic GVHD was higher in recipients with RR≥4.5% than in those with RR<4.5% (p= 0.0055). Using Cox proportional hazards model, the relative risk of chronic GVHD for patients with positive MLC (RR≥4.5%) was 2.54 (p=0.0019); for those who received PBPC as grafting was 2.92 (p=0.0002) and for patients who developed previous acute GVHD the risk was 2.27 (p=0.0361). When continuous RR values had been used, the relative risk of chronic GVHD increased 1.04 to each unit of RR (p=0.0049); for those who received PBPC the risk was 2.56 (p=0.0011) and for patients who developed previous acute GVHD the risk was 2.27 (p=0.0370). Our analysis showed that MLC was not useful to predict acute GVHD, but MLC with RR≥4.5% should be considered as a risk factor of chronic GVHD for HLA-identical sibling recipients. Thus, MLC reactivity associated to others risk factors can predict the development of chronic GVHD, helping in the prevention and/or treatment of this late complication of allogeneic BMT.

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VALACYCLOVIR FOR THE PREVENTION OF CYTOMEGALOVIRUS INFECTION AFTER ALLOGENEIC STEM CELL TRANSPLANTAION: A RETROSPECTIVE COHORT ANALYSIS

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Cytomegalovirus (CMV) infection (reactivation) is frequently encountered after allogeneic stem cell transplantation (SCT)

and is associated with significant post-transplant morbidity and mortality. Recent large prospective randomized studies using Valacyclovir for the prevention of CMV infection in renal transplant and AIDS patients have shown efficacy and safety. The benefit of this approach of CMV prophylaxis has not been reported in allogeneic SCT recipients. We hypothesized that Valacyclovir may be useful in the prevention of CMV infection after allogeneic SCT and performed a retrospective single center study was to evaluate our treatment approach. We compared a group of 31 patients who received Valacyclovir for CMV prophylaxis with a matched cohort of 31 patients who did not receive the drug or any other form of CMV prophylaxis. Valacyclovir was used as primary prophylaxis in 12 patients and as secondary prophylaxis (after a prior CMV reactivation was effectively treated with either Ganciclovir or Foscarnet and without CMV antigenemia at the start of Valacyclovir) in the remaining 19 patients. The two treatment groups were well matched for donor-recipient CMV serological-status and other pre-transplant characteristics. No patients died within the first 100 days of transplant. CMV reactivation was detected by blood antigenemia testing using a commercially available immunoflourescence assay for CMV early antigen pp65 on circulating leukocytes. When used as primary prophylaxis, 3/12 Valacyclovir patients had CMV reactivation compared to 24/31 control patients ($P < 0.001$). For secondary prophylaxis, 5/19 Valacyclovir patients reactivated compared to 16/24 control patients ($P < 0.05$). Valacyclovir was well tolerated except for infrequent and mild gastrointestinal side effects. Prophylaxis with Valacyclovir appears to be safe and efficacious in preventing both primary and secondary CMV reactivation after allogeneic SCT. Larger prospective randomized studies will be required to confirm these observations.

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NON-MYELOSUPPRESSIVE "MICRO-CONDITIONING" INDUCES ORGAN TRANSPLANT TOLERANCE AND CORRECTS HEMOGLOBINOPATHIES

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Stable donor hematopoietic chimerism eliminates the need for chronic immunosuppressive therapy following organ transplantation. Current experimental protocols to establish mixed chimerism employ myelosuppressive conditioning or very large "mega" doses of donor bone marrow. Using a single, low dose of busulfan, costimulatory blockade (CB, anti-CD40L mAb and CTLA4-Ig), and T-cell depleted bone marrow (TCD BM) transplantation, we have created "costimulation chimeras" that exhibit robust donor-specific tri-lineage tolerance (T, B, and NK cells). Animals treated with busulfan doses of 20 mg/kg or lower had no evidence of weight loss, hematopoietic toxicity, GvHD, or other signs of ill health. The percentage of donor cells in the peripheral blood on day +120 post-transplant among allogeneic recipients treated with 20 mg/kg busulfan and correlated directly with the engrafting dose of TCD BM ($2x10^7 \rightarrow$ 65%, $1x10^7 \rightarrow$ 57%, $5x10^6 \rightarrow$ 37%, $2x10^6 \rightarrow$ 26%), and were equivalent to the chimerism seen among mice receiving congenic TCD BM transplants following busulfan conditioning. Co-stimulation chimeras had long-term (>250 days) acceptance of skin allografts from the BM donor while rejecting third party grafts. Thalassemic mice that received allogeneic transplantation after "micro-conditioning" showed normalization of anemia and reticulocytosis without evident toxicity. The peripheral blood of costimulation chimeras showed increasing numbers of donor derived T-cells over time and deletion of the donor superantigen reactive Vβ11+ and Vβ5+ CD4+ T cells by day 60. Costimulation chimeras deleted Ly-49D+ NK cells that are reactive towards the (donor) but not the b (host) haplotype. Control groups (CB or TCD BM or busulfan alone) failed to delete donor reactive Vβ11+ or Vβ5+CD4+ T cells or Ly-49D+ NK cells supporting both peripheral as well as thymic deletion as a mechanisms for donor specific allograft tolerance.

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IL-17 INCREASES THE ENGRAFTMENT POTENTIAL OF HIGHLY PURIFIED CD34+ CELLS ON INDIVIDUAL STROMAL CELL COLONIES

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IL-17 is a novel cytokine produced by CD4+ T-cells and is beleived to facilitate lympho-hematopoietic stem cell engraftment. The number of blast cell colonies consisting of more than 50 blasts strongly adherent and nested to an individual stromal cell colony was used to measure the engrfatment potential of highly purified early hematopoietic CD34+ cells. Stromal-adherent CFU-BLAST derived colonies were measured at weekly intervals for 4 weeks post seeding of 1,500 purified (>95%)CD34+ cells per individual stromal cell colony. The number (M+/-SEM)blast cell nests of CD34+ cells were assessed on both pure fibroblast-like stromal cell colonies, CFU-F derived and mixed stromal cell colonies consisting of fibroblast-like cells and adipocytes, believed to be derived from multipotential stromal progenitors,CFU-FA.

Frequency of Blast cell colonies continuously increased similarly per CFU-F or CFU-FA from week 1=1+/-0.3, week 2=2+/-0.5, weeek 3=3+/-0.5, and week 4=3+/-0.4. In contrast, frequency of blast cell colonies was significantly higher with IL-17: CFU-BLAST/CFU-F at week 2=2+/-0.3, week 3=5+/-0.4,and week 4=5+/-0.6, and CFU-BLAST/CFU-FA at week 1=2+/-0.2 week 2=5+/-0.4, week 3=5+/-0.5, and week 4=6+/-0.3; (p=0.03 compared to control). IL-17 expanded CFU-BLAST colonization of CFU-F derived stromal cell colonies by 5X, and CFU-FA by 6X after 4 weeks post seeding of 1,500 CD34+ cells. However, cumulative production of CFU-BLASTS on both CFU-F and CFU-FA were similar in contriol cultures; 8 CFU-BLASTS/CFUF or CFU-FA. While IL-17 enhanced cumulative production to 13 CFU-BLAST/CFU-F(1.5X increase) and 17 CFU-BLAST/CFU-FA (>2X increase). The engraftment potential indirectly measured by frequency of CFU-BLASTS increased from 0.1% to 2.0% when seeded on individual stromal cell colonies.

These data indicate that IL-17 enhances the engrfatment potential of early CD34+ cells. These data suggest that IL-17 may facilitate engraftment by targeting mesenchymal/stromal progenitor derived "niches" to stimulate local expansion of early CD34+ hematopoietic stem cells.

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OUTCOME OF CHILDREN WHO UNDERGO STEM CELL TRANSPLANTATION (SCT) FOR "LATE RELAPSE" OF ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Late relapse of ALL in childhood, defined as relapse > 24 months from diagnosis is uncommon, but has been treated traditionally with intensive conventional chemotherapy. We report our experience for 12 patients (5M, 7F), with late relapse of ALL, who underwent SCT in CR2 rather than further conventional chemotherapy. Average age at diagnosis was 7.1 yrs (2-19, median 5) and at the time of SCT was 11 yrs (4.9-24, median 9.3) with CR1 duration of 41 mo (28-57, median 38). Stem cell sources were matched siblings (n=8), autologous peripheral blood cells (n=2), unrelated umbilical cord blood cells (n=1) and a syngeneic twin (n=1). The cytoreduction regimen was comprised of fractionated total body irradiation (150 x 8 = 1200 cGy), etoposide (1000 mg/m^2 infused over 24 hr) and cyclophosphamide (60 mg/kg x3). Supportive care included G-CSF in 8 and GM-CSF in one patient.

All patients engrafted with median time to an ANC > 500/ul of 14 days (10-52) and median time to an unsupported platelet count of 20,000/ul of 28 days (13-168). Toxicities included febrile neutropenia (n=10), bacteremia (n=1), acute graft-vs-host disease (GVHD), (Grade II, n=1, Grade III, n=2). Mucositis was present in all patients: Grade I, n=4; Grade II, n=6 and Grade III, n=2). Multi-organ system failure was fatal in one patient. Three other patients died from complications of aspergillus pneumonia (1),

sepsis (1) and combined marrow and CNS recurrence (1). Event-free-survival, with median follow-up of 33 mo (2-94) is 67%. We conclude SCT is an effective form of therapy for ALL patients suffering a "late relapse" of their disease. Additional follow-up will be required to confirm the efficacy of this approach for consolidating a second remission of childhood ALL.

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IL-12 EXHIBITS DIVERSE ROLES IN ACUTE GVHD

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Graft-versus-host disease (GVHD) remains a significant cause of morbidity following allogeneic bone marrow transplantation (BMT). The role of endogenous IL-12 is complicated by the production of two separate biologically active molecules from the subunits that comprise IL-12. IL-12 exists as a heterodimer consisting of p35 and p40 chains, p40 homodimer has been reported to be an antagonist for the heterodimer. IL-12 knockout (KO) mice of either p35 or p40 chains were used as either donors and/or recipients in an allogeneic (total MHC mismatch) bone marrow transplant model of GVHD. The p35 KO mouse can produce the p40 homodimer whereas the p40 KO mouse lacks both IL-12 and p40 homodimer. When IL-12 p35 KO mice were used as recipients, they succumbed quicker from acute GVHD (as determined by survival, weight loss, and histological assessment) whereas the IL-12 p40 KO recipients demonstrated some protection from GVHD compared to control wild-type mice recipients. Despite the absence of donor and recipient sources of IL-12 in p35 KO recipients, serum IFN-γ was significantly higher in p35KO recipients compared to IL-12 wild type recipients on day 3 post-BMT. These results demonstrate that the p40 homodimer in the p35 KO mice accelerates GVHD whereas the total absence of IL-12 is mildly protective. The results suggest that IL-12 heterodimer may have both immuno-suppressive and immuno-stimulatory properties in this murine model of acute GVHD.

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AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH ACUTE MYELOGENOUS LEUKEMIA IN FIRST COMPLETE REMISSION

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Background: Early autologous stem cell transplantation (ASCT) in patients with acute myelogenous leukemia (AML) is increasingly used as post remission therapy in the last years.

Patients and methods: Seventeen patients (7 male, 10 female, age: 18-69) were treated with idarubicin (12 mg/m^2, d1-3), cytosine arabinoside (200 mg/m^2,d1-5) as induction and cytosine arabinoside (2000mg/m^2, twice a day, d1-3), mitoxantrone (5 mg/m^2,twice a day, d1-3)as continuous infusion for consolidation therapy. The stem cell harvest was performed after the first or second consolidation therapy under stimulation with G-CSF starting on day +7. The median CD34+ count was 6.1 x 10^6 cells/kg BW (range 3.2-23). The mean time from diagnosis to ASCT was 6 (range: 4-12) months. Conditioning regimen consisted of fTBI (6 x 2 Gray) and cyclophosphamide (2 x 60 mg/kg BW).

Results: The median time to engraftment (ANC > 0.5 x 10^9 /L) was on day +12. After a mean observation time of 21 months the 4 years probability of relapse-free survival (Kaplan-Meier) is 61%. We conclude that early ASCT in first complete remission is an effective treatment.